

Sghrt polypeptide and related products, methods and uses

TECHNICAL FIELD

5 The present disclosure relates broadly to a polypeptide and related products, methods and uses.

BACKGROUND

10 Heart failure (HF) remains a leading cause of death and one of the costliest healthcare burdens in modern society. In Singapore, heart disease accounted for 19.3% of all deaths in 2014 (<http://www.myheart.org.sg/article/about-the-heart-and-heart-disease/statistics/singapore/75>). Treatment for HF remains an inadequate stage-by-stage cumulative add-on therapy, and morbidity and
15 mortality threaten to worsen as the world population rapidly ages.

 The underlying cause of HF in most patients is a loss of cardiomyocytes, accompanied by functional derangements in contraction and relaxation. The regenerative capacity of the heart is limited by the inability of terminally differentiated cardiomyocytes to adequately undergo cell division after the first
20 weeks of life.

 While recent studies have found that very low rate of cardiomyocyte turnover occurs in adult mouse and human hearts mediated primarily by proliferation of pre-existing cardiomyocytes, the drivers of the proliferation remain elusive. Novel targets, especially novel peptidic targets that can be manipulated
25 to drive proliferation of adult cardiomyocytes, are urgently needed for the HF drug discovery pipeline.

 Thus, there is a need to provide a polypeptide and related products, methods and uses that address or at least ameliorate one or more of the above problems.

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SUMMARY

In one aspect, there is provided an isolated polypeptide encoded by Sghrt.

In one embodiment, the polypeptide comprises a mitochondrial
5 polypeptide.

In one embodiment, the polypeptide shares at least 75% sequence identity with any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 18, and SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25.

10 In one embodiment, the polypeptide comprises a micropeptide.

In one embodiment, the polypeptide comprises a homo-oligomer.

In one aspect, there is provided a method of assessing heart function in a subject, the method comprising: determining an expression level of the polypeptide in a sample obtained from the subject; comparing the expression
15 level to a reference expression level, wherein if the expression level exceeds the reference expression level, the subject is considered to have impaired or deteriorated heart function.

In one embodiment, the reference expression level comprises an expression level of the polypeptide in a healthy population.

20 In one embodiment, the reference expression level comprises an expression level of the polypeptide in an earlier sample obtained from the subject.

In one embodiment, the method further comprises administering to the subject an inhibitor of the polypeptide.

In one aspect, there is provided a method of treating impaired heart
25 function in a subject, the method comprising: inhibiting an expression of the polypeptide in the subject.

In one aspect, there is provided a method of identifying a potential drug for treating impaired heart function, the method comprising: determining a first expression level of the polypeptide in a cell; exposing the cell to a drug candidate;
30 and determining a second expression level of the polypeptide after the exposure, wherein if the second expression level is lower than the first expression level,

then the drug candidate is identified as a potential drug for treating impaired heart function.

In one aspect, there is provided an inhibitor of the polypeptide.

In one aspect, there is provided the inhibitor for use in therapy.

5 In one aspect, there is provided the inhibitor for use in treating impaired heart function.

In one aspect, there is provided use of the inhibitor in the manufacture of a medicament for the treatment of impaired heart function.

In one embodiment, the impaired heart function is selected from the group
10 consisting of: myocardial infarction, heart failure, coronary artery disease, narrowing of the arteries, heart attack, abnormal heart rhythms, arrhythmias, heart failure, heart valve disease, congenital heart disease, heart muscle disease, cardiomyopathy, pericardial disease, aorta disease, marfan syndrome, genetic cardiomyopathy, non-genetic cardiomyopathy, heart hypertrophy,
15 pressure overload-induced heart dysfunction, and damaged heart tissue.

In one aspect, there is provided a pharmaceutical composition comprising the inhibitor and a suitable carrier, adjuvant, diluent and/or excipient.

In one aspect, there is provided a vector comprising a polynucleotide sequence encoding for the polypeptide or the inhibitor.

20 In one aspect, there is provided a host cell transfected with the vector.

In one aspect, there is provided a transgenic non-human subject comprising a polynucleotide construct encoding for the polypeptide.

In one aspect, there is provided the polypeptide coupled to a detectable label.

25 In one aspect, there is provided a method of dedifferentiating and/or proliferating a heart cell, the method comprising: inhibiting the expression of the polypeptide in the heart cell.

In one aspect, there is provided a cell produced by the method, or progenies or cell derivatives thereof.

30 In one aspect, there is provided an isolated polynucleotide encoding for the polypeptide.

DEFINITIONS

The term "polypeptide" as used herein broadly refers to a chain of amino acid residues connected via peptide bonds. Further, the term also encompasses
5 an assembly or a complex of more than one polypeptide subunit, such as multimers or oligomers. In some embodiments, the multimers or oligomers are homo-multimers or homo-oligomers composed of identical subunits. The polypeptide may be naturally occurring or synthetic (e.g., generated by chemical synthesis or recombinant DNA technology). Examples of "polypeptide" include
10 gene products, naturally-occurring peptide/proteins, homologs, orthologs, paralogs, fragments, and other equivalents, variants, analogs, multimeric or oligomeric forms of the foregoing. No particular size is implied by the term "polypeptide".

The term "isolated" as used herein in relation to a polypeptide refers to a
15 polypeptide that is removed from its natural environment. A polypeptide may be "isolated" by separating it from some or all of the naturally occurring constituents with which it is associated in nature. Thus, an "isolated" polypeptide is typically at least partially purified.

The term "expression" as used herein in relation to a polypeptide is not
20 limited to an amount of the polypeptide, but also includes the meaning of "functional expression" or an activity of the polypeptide to perform a native function. Accordingly, "determining an expression level" of a polypeptide includes measuring quantitatively, semi-quantitatively or qualitatively the amount of the polypeptide and/or an activity of the polypeptide.

25 The term "treatment", "treat" and "therapy", and synonyms thereof as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a medical condition, which includes but is not limited to diseases (such as heart diseases or impaired heart function), symptoms and disorders. A medical condition also
30 includes a body's response to a disease or disorder, e.g. inflammation. Those in need of such treatment include those already with a medical condition as well as

those prone to getting the medical condition or those in whom a medical condition is to be prevented.

The term "therapeutically effective amount" of an agent will be an amount of the active agent that is capable of preventing or at least slowing down (lessening) a medical condition, such as heart diseases or impaired heart function. Dosages and administration of agents, compounds, compositions and formulations of the present disclosure may be determined by one of ordinary skill in the art of clinical pharmacology or pharmacokinetics. See, for example, Mordenti and Rescigno, (1992) *Pharmaceutical Research*. 9:17-25; Morenti et al., (1991) *Pharmaceutical Research*. 8:1351-1359; and Mordenti and Chappell, "The use of interspecies scaling in toxicokinetics" in *Toxicokinetics and New Drug Development*, Yacobi et al. (eds) (Pergamon Press: NY, 1989), pp. 42-96. An effective amount of the active agent of the present disclosure to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it may be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect.

The term "subject" as used herein includes patients and non-patients. The term "patient" refers to individuals suffering or are likely to suffer from a medical condition e.g. a heart condition/disorder and/or impaired heart function, while "non-patients" refer to individuals not suffering and are likely to not suffer from the medical condition. "Non-patients" include healthy individuals, non-diseased individuals and/or an individual free from the medical condition. The term "subject" includes humans and animals. Animals include murine and the like. "Murine" refers to any mammal from the family Muridae, such as mouse, rat, and the like.

The term "micro" as used herein is to be interpreted broadly to include dimensions from about 1 micron to about 1000 microns.

The term "nano" as used herein is to be interpreted broadly to include dimensions less than about 1000 nm.

The term "particle" as used herein broadly refers to a discrete entity or a discrete body. The particle described herein can include an organic, an inorganic

or a biological particle. The particle used described herein may also be a macro-particle that is formed by an aggregate of a plurality of sub-particles or a fragment of a small object. The particle of the present disclosure may be spherical, substantially spherical, or non-spherical, such as irregularly shaped particles or ellipsoidally shaped particles. The term "size" when used to refer to the particle broadly refers to the largest dimension of the particle. For example, when the particle is substantially spherical, the term "size" can refer to the diameter of the particle; or when the particle is substantially non-spherical, the term "size" can refer to the largest length of the particle.

10 The terms "coupled" or "connected" as used in this description are intended to cover both directly connected or connected through one or more intermediate means, unless otherwise stated.

 The term "associated with", used herein when referring to two elements refers to a broad relationship between the two elements. The relationship includes, but is not limited to a physical, a chemical or a biological relationship. For example, when element A is associated with element B, elements A and B may be directly or indirectly attached to each other or element A may contain element B or vice versa.

 The term "adjacent" used herein when referring to two elements refers to one element being in close proximity to another element and may be but is not limited to the elements contacting each other or may further include the elements being separated by one or more further elements disposed therebetween.

 The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either meaning.

 Further, in the description herein, the word "substantially" whenever used is understood to include, but not restricted to, "entirely" or "completely" and the like. In addition, terms such as "comprising", "comprise", and the like whenever used, are intended to be non-restricting descriptive language in that they broadly include elements/components recited after such terms, in addition to other components not explicitly recited. For example, when "comprising" is used, reference to a "one" feature is also intended to be a reference to "at least one" of

that feature. Terms such as "consisting", "consist", and the like, may in the appropriate context, be considered as a subset of terms such as "comprising", "comprise", and the like. Therefore, in embodiments disclosed herein using the terms such as "comprising", "comprise", and the like, it will be appreciated that

5 these embodiments provide teaching for corresponding embodiments using terms such as "consisting", "consist", and the like. Further, terms such as "about", "approximately" and the like whenever used, typically means a reasonable variation, for example a variation of +/- 5% of the disclosed value, or a variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a

10 variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and teaches all possible sub-ranges as well as individual numerical

15 values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values within that range such as 1%, 2%, 3%, 4% and 5%. The intention of the above specific disclosure is

20 applicable to any depth/breadth of a range.

Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other

25 sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required, a method and/or process disclosed herein should not be limited to the steps being carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

30 Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be

disclaimed in other alternative embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

DESCRIPTION OF EMBODIMENTS

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Exemplary, non-limiting embodiments of a polypeptide, and related products, methods and uses are disclosed hereinafter.

In various embodiments, there is provided a peptide encoded by *Sghrt*, or a part/fragment thereof. In some embodiments, the peptide comprises an isolated
10 peptide. In some embodiments, the peptide comprises an oligopeptide. In some embodiments, the peptide comprises a polypeptide. In some embodiments therefore, there is provided an isolated polypeptide encoded by *Sghrt*. In various embodiments, *Sghrt* corresponds to the previously annotated 1810058i24 *Rik*, or homologs or orthologs thereof.

15 In various embodiments, the peptide comprises a secretory peptide.

In various embodiments, the polypeptide comprises no more than about 150, no more than about 140, no more than about 130, no more than about 120, no more than about 110, no more than about 100, no more than about 90, no more than about 80, no more than about 70, no more than about 60, no more
20 than about 55, no more than about 50, no more than about 49, no more than about 48, or no more than about 47 amino acids in length. In various embodiments, the polypeptide comprises from about 40 to about 50, or from about 45 to about 50 amino acids in length. In various embodiments, the polypeptide comprises about 45, about 46, about 47, about 48, about 49 or about
25 50 amino acids in length. In one embodiment, the polypeptide comprises about 47 amino acids in length. In some embodiments, the polypeptide comprises a short open reading frames-encoded (sORF-encoded) peptide. In some embodiments, the polypeptide comprises a micropeptide.

In various embodiments, the polypeptide is capable of oligomerization or
30 capable of forming multimers or oligomers. In various embodiments, the polypeptide is capable of forming homo-multimers or homo-oligomers. In various embodiments therefore, the polypeptide comprises a multimer, an oligomer or a

polypeptide assembly or complex. The polypeptide may be multimer or an oligomer composed of at least about two or at least about three polypeptide subunits or identical polypeptide subunits. In some examples, the polypeptide may be a dimer (e.g. a homodimer), a trimer (e.g. a homotrimer), and in some
5 examples, the polypeptide may be monomer (e.g. composed of no more than one polypeptide subunit). The multimeric or oligomeric forms of the polypeptide may be substantially resistant to heat/high temperature and/or substantially resistant to treatment with ethylenediaminetetraacetic acid (EDTA). In various embodiments, the monomers or polypeptide subunits of the multimer or oligomer
10 are not linked/joined by covalent bond.

In various embodiments, the polypeptide is located in, or localizes to, the mitochondria in a cell, optionally wherein the polypeptide is predominantly located in the mitochondria in a cell. In various embodiments, the polypeptide comprises a mitochondrial polypeptide. In various embodiments, the polypeptide comprises
15 a sORF-encoded mitochondrial polypeptide. In various embodiments, the polypeptide comprises a mitochondria membrane protein. In some embodiments, the polypeptide comprises a mitochondrial targeting signal, optionally a mitochondrial membrane targeting signal.

The polypeptide may be located in, or may localize to, or may be found in
20 the mitochondrial matrix or the intermembrane of mitochondria. The polypeptide may also be located in, or may localize to, or may be found in the cell cytoplasm, nucleus or extracellular region etc.

In some embodiments, the polypeptide is capable of entering a secretory pathway. In some embodiments, the polypeptide comprises a secretory signal sequence, optionally wherein the secretory signal sequence comprises
25 "MLQFLLGFTLGNVVGMYLE" or portions (e.g. linear portions) thereof, or a sequence having at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at
30 least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at

least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identity thereto. The secretory signal sequence may be from about 15 to about 25 amino acids long or from about 17 to about 23 amino acids long. The secretory signal sequence may be about 17, about 18, about 19, about 20, about 21, about 22 or about 23 amino acids long.

In some examples, based on a hydropathy analysis of the sequence "MLQFLLGFTLGNVVGMYLE", the sequence more likely constitutes a secretory signal sequence than a mitochondrial signal sequence.

The mitochondrial targeting signal sequence and/or the secretory signal sequence of the polypeptide may be cleaved off to produce a C-terminal product that is from about 20 to about 30 amino acids long, or from about 25 to about 30 amino acids long. The C-terminal product may be about 25, about 26, about 27, about 28, about 29 or about 30 amino acids long. In various embodiments, the C-terminal product comprises the sequence "QNYDIPNLAKKLEEIKKDLDAKKKPPSA" or portions (e.g. linear portions) thereof or a sequence having at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78% or at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identity thereto.

In various embodiments, the polypeptide or a part/fragment thereof shares at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78% or at least about 79%, at least about 80%,

at least about 81%, at least about 82%, at least about 83%, at least about 84%,
at least about 85%, at least about 86%, at least about 87%, at least about 88%,
at least about 89%, at least about 90%, at least about 91%, at least about 92%,
at least about 93%, at least about 94%, at least about 95%, at least about 96%,
5 at least about 97%, at least about 98%, at least about 99% identity or about 100%
identity with a sequence set forth in any one of:

SEQ ID NO: 1:

MLQFLLGFTLGNVVGMYLAQNYDIPNLAKKLEEIKKDLDAKKKPPSA;

SEQ ID NO: 2:

10 MLQFLLGFTLGNVVGMYLAQNYDIPNLAKKLEEDIKKDLDAKKKPPSS;

SEQ ID NO: 3:

MLQFLLGFTLGNVVGMYLAQNYDIPNLAKKLEEIKKDLDAKKKPPSC;

SEQ ID NO: 4:

MLQFLLGFTLGNVVGMYLAQNYDMPNLAKKLEEIKKDLDAKKKPPSS;

15 SEQ ID NO: 5:

MLQFLLGFTWGNVVGMYLAQNYEMPNLAKKLEEIKKDLEAKKKPPSS;

SEQ ID NO: 6:

MLQFVLGFTLGNVVGMYLAQNYDIPNIAKKLEDFKKDVEAKKKPPSDKS;

SEQ ID NO: 7:

20 MMQFILGFTLGNVVGMYLAQNYEVPNISKKIEAFKKDVEAKKKPPE;

SEQ ID NO: 18:

MLQFLLGFTLGNVVGMYLA;

SEQ ID NO: 19:

MXQFXLGFTXGNVVGMYLAQNYXXPNXXXKKXEXXKKDXXAKKKPPX, wherein

25 X represents any amino acid;

SEQ ID NO: 20:

GNVVGMYLAQNY

SEQ ID NO: 21:

AKKKPP;

30 SEQ ID NO: 22:

MLQFLLGFTLGNVVGMY;

SEQ ID NO: 23:

QNYDIPNLAKKLEEIKKDLDAKKKPPSA;

SEQ ID NO: 24:

DIP;

SEQ ID NO: 25:

5 KDLDAKKKPPSA;

and

the sequence of short transmembrane mitochondrial protein 1 precursor (STMP1) or

portions, optionally linear portions, thereof.

10 In some embodiments, the polypeptide shares at least 75% sequence identity with any one of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25 and portions, optionally linear portions, thereof.

15 In some embodiments, the polypeptide or a part/fragment thereof comprises the sequence “MXQFXLGFTXGNVVGMVLAQNYXXPNXXXKKXEXXKKDXXAKKKPPX” (SEQ ID NO: 19), or portions, optionally linear portions, thereof, wherein X represents any amino acid. It will be appreciated that each of X may represent different
20 amino acids. In some embodiments, each of X is independently selected from the group consisting of A, R, N, D, C, E, Q, G, H, I, L, K, M, F, P, S, T, W, Y and V. In some embodiments, each of X is independently selected from the group consisting of A, D, E, F, I, L, S, M, V and W. In some embodiments, the polypeptide or a part/fragment thereof comprises the sequence
25 “GNVVGMVLAQNY” (SEQ ID NO: 20) and/or “AKKKPP” (SEQ ID NO: 21), or portions, optionally linear portions, thereof. In some embodiments, the polypeptide or a part/fragment thereof comprises the sequence “MLQFLLGFTLGNVVGMV” (SEQ ID NO: 22) or portions, optionally linear portions, thereof.

30 In some embodiments, the polypeptide or a part/fragment thereof is derived, isolated or purified from a mammal, optionally a mammal selected from human, pig, rat and mouse. In some examples, the presence of the polypeptide

or a part/fragment thereof in human, pig, rat and mouse has been confirmed by mass spectrometry.

In various embodiments, the polypeptide is encoded by a polynucleotide or nucleic acid sequence (DNA/RNA) that shares at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 100% sequence identity with the sequences set forth in any one of SEQ ID No: 8 to SEQ ID No: 15.

In various embodiments, there is provided an isolated polynucleotide or nucleic acid sequence (DNA/RNA) encoding for the polypeptide or a part/fragment thereof. In various embodiments, there is provided an isolated polynucleotide or nucleic acid sequence encoded by *Sghrt*. In various embodiments, the polynucleotide or nucleic acid sequence shares at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or at least about 100% sequence identity with the sequences set forth in any one of SEQ ID No: 8 to SEQ ID No: 15.

In various embodiments, the isolated polynucleotide or nucleic acid sequence comprises a human *Sghrt* RNA sequence. In various embodiments,

the human Sghrt RNA sequence comprises the sequence set forth in SEQ ID NO:14: GACCGGCCGCGGAGCTGCTGCAGTCCTTCGCGCCCTCCTCGCC CTCCCCACCGACATCATGCTCCAGTTCCTGCTTGGATTTACACTGGGCAAC GTGGTTGGAATGTATCTGGCTCAGAACTATGATATACCAAACCTGGCTAAA

5 AAACCTGAAGAAATTA AAAAGGACTTGGATGCCAAGAAGAAACCCCCTAGT GCATGAGACTGCCTCCAGCACTGCCTTCAGGATATACTGATTCTACTGCTC TTGAGGGCCTCGTTTACTATCTGAACCAAAGCTTTTGTTCGTCTCCAG CCTCAGCACTTCTCTTCTTTGCTAGACCCTGTGTTTTTGTCTTAAAGCAAG CAAAATGGGGCCCCAATTTGAGA ACTACCCGACATTTCCAACATACTCACC

10 TCTTCCCATAATCCCTTTCCA ACTGCATGGGAGGTTCTAAGACTGGAATTA TGGTGCTAGATTAGTAAACATGACTTTTAATGAGTAGTGTCTTCTTTATCGT TTGCGATTTTTACTACCTTTTTTCAAAGAAAAATTGATGAGTTTGTATAGC TGGTCAGATACAAATAATAGTGACTTCACAGTTTAGTAATTATAATGGGTAC TTGTTAAACATTTGGTACTAAATTATGTTGCTGCAAAGTAATTA AATTAGTA

15 TCTAGAGCTAGTTTCTGGTGAATTATTCATTTATTTTGTACTGTTGTTAGGC AGCTCTGTAGTTGCTAATTTAACCAATAAGTCAATTTGCTATTCATGAAGAA ACGATTCTGAGAATCCTGTCAGGAATTGGGGAATGAAAAAATACACAAAAT AATGGTCTTTGTCCCAGTAGAGTTCATAGTCTATTTAGTGTGCATGTTTTTC CTTAATGATGTATTTGATCTGACTTTTTTCTTCTCAAAGAATCATACTTGG

20 GATTACAGGTACATTTGATGTTATATGATGGATAAGTGAAAAGTTTTTAAAG GAGATTTTATACCTTTTCACATTAAAAAAGGTATTTATATTACTTTGTAG TGATTGTCTTAAGAAAAAATATAGCCCAAATGTATAGTAAATCAGCAGCTC AAGAAGAATTTCTGCTTCTCTTTGTAGTTGATGCTTTGTTTTTCTGTCAGT CAGAAATTCCTTGATTTTGTCAAATGTATAATCAGCTTGATTGTTTTTAAAT

25 TAAAAAAAATTTGAATAATTA ACTTTTGCCATGGGACAAGATACAAAAGTA ATTTCATATAAAGGGCCTCTCCCACCCCTGTTCTCTGGCTCCTGGCTCCTG TTTGACAAGTTACTGTTACCACTTCGCCTTATACTTTTGAGAAAGAGTCTGT GCCTAAACAAACACGTGTAACACAAATAGTAACTATACATGGAGGTCTAGC CCTCGCCTTTTTTTTTTCTTTTTTCTTTTTTAATGGAGATCATTCTATACCA

30 GCATGTAAGTAGCAAGGAACCTCATTCTTTTTTGGCTGCCTAAAATTTTTT TGAATAGATATAACATAATTGATTTAATCTGCTACTGGTGAATGCTTAGGTT GTTCTTTTGTCTATTACAGTGATAACTTCAATCCTAATGTTATTAAGCATATCG

ATTCAGGGTATAGCTATAAGATGAAGTCCTAAAAGTATAATTTAGACTAAAT
ACAAATACCCATTTTCGCTAGCTGTTTTGTTTCAGAGGACTTGTTGAGCAGC
TTCATAATAATGCCATTTTTGAAGACATGGCAGGTTTCAAGTCAATAAACT
GGAAGAATTGTTTCAGAGCATCTTTTTTCAGACAGTGATGACATTGATTCTGT
5 ATATGATAAAGTGATTCTGCTTCTCTTTGACAACTTGCATCTCTCCTACATG
GAAGTAAGTTTTATTCCTGTCAATGTTGTCTTTGTGTGTGACAGATTAGGAT
TAAATTATGGTTTGACTTTTCCTAGCAGCGTGATCATGGGCAAGTGGCTTT
TTTTTTTTTTTTTTTTTGGAGACAGAGTCTCACTCTGCTGCCCAGGCTGGAGT
GCAGTGGCACAGTCTTGGCTCACTGCAACTCCTGCCTCCCGGTCCAAGTG
10 ATTCTCGTGCTGCAGCTTCTCAAGTAGCTGGCATCACCACCACACCTGGCT
AATTTTTGTATTTTTAGTAACGACGAGGTTTCACCATGTTGGCAAAGCTGGT
CTCAAATTCCTGGCCTCAAGTGATCTGCCCACTTCAGCCTCCCAAAGTGTT
GGGATTACAGGCGTGAGCCACTGCGCCCAGCTTTTTTAACTTTTAGATTCT
ATTTAATAGGTAAATTGCATGTCACGGGTTTGTAGCTTATTCTTTCAGAAAC
15 TCTTGCATTATCTGTAGACGTGGACGTAAATATCCACCTCATAGGGTTTTCA
TAAAAAATAATTGAGATAATGTATGTAATGTTTCACAGTGCTTTCAGACTA
TCTAATAAATAGTAGCTATTAGTA. In some embodiments, the isolated
polynucleotide or nucleic acid sequence comprises a coding region (or CDS) of
a human Sghrt RNA sequence. In various embodiments, the CDS comprises the
20 sequence set forth in SEQ ID NO:15: ATGCTCCAGTTCCTGCTTGGATTTACA
CTGGGCAACGTGGTTGGAATGTATCTGGCTCAGAACTATGATATACCAAAC
CTGGCTAAAAAACTTGAAGAAATTAAGAAAGGACTTGGATGCCAAGAAGAAA
CCCCCTAGTGCATGA. In some embodiments, the isolated polynucleotide or
nucleic acid sequence comprises a 5' untranslated region (5'UTR) of a human
25 Sghrt RNA sequence. In various embodiments, the 5'UTR of the human Sghrt
RNA sequence comprises the sequence set forth in SEQ ID NO:16: GACCGG
CCCGCGGAGCTGCTGCAGTCCTTCGCGCCCTCCTCGCCCTCCCCACCGA
CATC. In some embodiments, the isolated polynucleotide or nucleic acid
sequence comprises a 3' untranslated region (3'UTR) of a human Sghrt RNA
30 sequence. In various embodiments, the 3'UTR of the human Sghrt RNA
sequence comprises the sequence set forth in SEQ ID NO:17:
GACTGCCTCCAGCACTGCCTTCAGGATATACTGATTCTACTGCTCTTGAGG

GCCTCGTTTACTATCTGAACCAAAAGCTTTTGTTCGTCTCCAGCCTCAG
CACTTCTCTTCTTTGCTAGACCCTGTGTTTTTTGCTTTAAAGCAAGCAAAAT
GGGGCCCCAATTTGAGAACTACCCGACATTTCCAACATACTCACCTCTTCC
CATAATCCCTTTCCAAGTGCATGGGAGGTTCTAAGACTGGAATTATGGTGC
5 TAGATTAGTAAACATGACTTTTAATGAGTAGTGTCTTCTTTATCGTTTGCGA
TTTTTACTACCTTTTTTCAAAAGAAAAATTGATGAGTTTTGTATAGCTGGTCA
GATACAAATAATAGTGAAGTTCACAGTTTAGTAATTATAATGGGTACTTGTTA
AACATTTGGTACTAAATTATGTTGCTGCAAAGTAATTAATAATTAGTATCTAG
AGCTAGTTTCTGGTGAATTATTCATTTATTTTGTACTGTTGTTAGGCAGCTC
10 TGTAGTTGCTAATTTAACCAATAAGTCAATTTGCTATTCATGAAGAAACGAT
TCTGAGAATCCTGTCAGGAATTGGGGAATGAAAAAATACACAAAATAATGG
TCTTTGTCCCAGTAGAGTTCATAGTCTATTTAGTGTGCATGTTTTTCCTTAA
TGATGTATTTGATCTGACTTTTTTCTTCTCAAAAGAATCATACTTGGGATTA
CAGGTACATTTGATGTTATATGATGGATAAGTGAAAAGTTTTTAAAGGAGAT
15 TTTATACCTTTTACATTAAAAAAGGTATTTATATTACTTTGTAGTGATT
GTCTTAAGAAAAAATATAGCCCAAATGTATAGTAAAATCAGCAGCTCAAGAA
GAATTTCTGCTTCTCTTTGTAGTTGATGCTTTGTTTTTCTGCAGTCAGAA
ATTCCTTGATTTGTCAAATGTATAATCAGCTTGATTGTTTTTAAATTAATA
AAAAATTTGAATAATTAAGTCTTTGCCATGGGACAAGATACAAAAGTAATTTT
20 ATATAAAGGGCCTCTCCCACCCCTGTTCTCTGGCTCCTGGCTCCTGTTTGA
CAAGTTACTGTTACCACTTCGCCTTATACTTTTGAGAAAGAGTCTGTGCCTA
AACAAACACGTGTAACACAAATAGTAAGTATACATGGAGGTCTAGCCCTCG
CCTTTTTTTTTTCTTTTTTCTTTTTTAATGGAGATCATTCTATACCAGCATGT
AAGTAGCAAGGAACCTCATTCTTTTTTGGCTGCCTAAAATTTTTTGAATA
25 GATATAACATAATTGATTTAATCTGCTACTGGTGAATGCTTAGGTTGTTCTT
TTGCTATTACAGTGATAACTTCAATCCTAATGTTATTAAGCATATCGATTCA
GGGTATAGCTATAAGATGAAGTCCTAAAAGTATAATTTAGACTAAATACAAA
TACCCATTTTCGCTAGCTGTTTTGTTTCAGAGGACTTGTTGAGCAGCTTCAC
TAATAATGCCATTTTTGAAGACATGGCAGGTTTCAAGTCAATAAACTGGAA
30 GAATTGTTTCAGAGCATCTTTTTTCAGACAGTGATGACATTGATTCTGTATAT
GATAAAGTGATTCTGCTTCTCTTTGACAACTTGCATCTCTCCTACATGGAAG
TAAGTTTTATTCTGTCAATGTTGTCTTTGTGTGTGACAGATTAGGATTAAA

TTATGGTTTGACTTTTCCTAGCAGCGTGATCATGGGCAAGTGGCTTTTTTTTT
 TTTTTTTTTTTTGGAGACAGAGTCTCACTCTGCTGCCCAGGCTGGAGTGCAG
 TGGCACAGTCTTGGCTCACTGCAACTCCTGCCTCCCGGTCCAAGTGATTC
 TCGTGCTGCAGCTTCTCAAGTAGCTGGCATCACCACCACACCTGGCTAATT
 5 TTTGTATTTTTAGTAACGACGAGGTTTCACCATGTTGGCAAAGCTGGTCTC
 AAATTCCTGGCCTCAAGTGATCTGCCCACTTCAGCCTCCCAAAGTGTTGGG
 ATTACAGGCGTGAGCCACTGCGCCCAGCTTTTTTAACTTTTAGATTCATTT
 AATAGGTAAATTGCATGTCACGGGTTTGTAGCTTATTCTTTCAGAACTCTT
 GCATTATCTGTAGACGTGGACGTAAATATCCACCTCATAGGGTTTTTCATAA
 10 AAAATAATTGAGATAATGTATGTAATGTTTCACAGTGCTTTGCAGACTATCT
 AATAAATAGTAGCTATTAGTA.

In various embodiments, the polypeptide comprises a human Sghrt
 secreted peptide or a part/fragment thereof. In various embodiments,
 the human Sghrt secreted peptide comprises the sequence set forth
 15 in SEQ ID NO:18: MLQFLLGFTLGNVVGMYLA or SEQ ID NO: 22:
 MLQFLLGFTLGNVVGMY or portions, optionally linear portions, thereof.

In various embodiments, the polypeptide, the polynucleotide or the nucleic
 acid sequence or a part/fragment thereof shares at least about 65%, at least
 about 66%, at least about 67%, at least about 68%, at least about 69%, at least
 20 about 70%, at least about 71%, at least about 72%, at least about 73%, at least
 about 74%, at least about 75%, at least about 76%, at least about 77%, at least
 about 78%, at least about 79%, at least about 80%, at least about 81%, at least
 about 82%, at least about 83%, at least about 84%, at least about 85%, at least
 about 86%, at least about 87%, at least about 88%, at least about 89%, at least
 25 about 90%, at least about 91%, at least about 92%, at least about 93%, at least
 about 94%, at least about 95%, at least about 96%, at least about 97%, at least
 about 98%, at least about 99% or at least about 100% sequence identity with the
 sequences indicated in Table 1 below, or portions, optionally linear portions,
 thereof.

30

Table 1

SEQ ID NO	
1	Human Sghrt peptide sequence / Homo sapien Sghrt peptide sequence / Chimpanzee Sghrt peptide sequence / Pan troglodytes Sghrt peptide sequence/ Bonobo Sghrt peptide sequence / Pan paniscus Sghrt peptide sequence
2	Pig Sghrt peptide sequence / Sus scrofa Sghrt peptide sequence
3	Cow Sghrt peptide sequence / Bos taurus Sghrt peptide sequence
4	Rat Sghrt peptide sequence / Rattus norvegicus peptide sequence
5	Mouse Sghrt peptide sequence / Mus musculus peptide sequence
6	Chicken Sghrt peptide sequence / Gallus gallus peptide sequence
7	Zebrafish Sghrt peptide sequence / Danio rerio peptide sequence
8	Mouse Sghrt RNA (ENSMUST00000152147)
9	Mouse Sghrt RNA sequence (ENSMUST00000136110)
10	Mouse Sghrt RNA sequence (ENSMUST00000130875)
11	Mouse Sghrt DNA sequence
12	Human Sghrt DNA sequence
13	Human Sghrt RNA sequence
14	Human Sghrt RNA sequence (5'UTR-CDS-3'UTR)
15	Human Sghrt RNA sequence (CDS only)
16	Human Sghrt RNA sequence (5'UTR only)
17	Human Sghrt RNA sequence (3'UTR only)
18	Human Sghrt peptide sequence 2
19	Shared Sghrt peptide sequence 1: MXQFXLGFTXGNVVGMYLAQNYXXPNXXXKKXEXXKKDXXAKKKPPX
20	Shared Sghrt peptide sequence 2: GNVVGMYLAQNY
21	Shared Sghrt peptide sequence 3: AKKKPP
22	Human Sghrt peptide sequence 3: MLQFLLGFTLGNVVGMY
23	Predicted C-terminal product: QNYDIPNLAKKLEEIKKDLDAKKKPPSA
24	Predicted antigenic residue 1: DIP
25	Predicted antigenic residue 2: KDLDAAKKKPPSA

In various embodiments, the size/molecular weight/molecular mass of the polypeptide or a part/fragment thereof is from about 1 KDa to about 30 KDa, from about 5 KDa to about 20 KDa, from about 5 KDa to about 15 KDa, from about 5 KDa to about 12 KDa or from about 15 KDa to about 20 KDa. In some embodiments, the size/molecular weight/molecular mass of the polypeptide is from about 5 KDa to about 6 KDa, or about 5kDa, about 5.8 KDa or about 6 KDa. In some embodiments, the size/molecular weight/molecular mass of the polypeptide in its monomeric form is from about 5 KDa to about 6 KDa, or about

5 KDa, about 5.8 KDa or about 6 KDa. In some embodiments, the size/molecular weight/molecular mass of the polypeptide or a part/fragment thereof is from about 7 KDa to about 9 KDa, or about 7 KDa, about 8 KDa or about 9 KDa. In some embodiments, the size/molecular weight/molecular mass of the polypeptide in its dimeric form is from about 7 KDa to about 9 KDa, or about 7 KDa, about 8 KDa or about 9 KDa. In some embodiments, the size/molecular weight/molecular mass of the polypeptide or a part/fragment thereof is from about 11 KDa to about 13 KDa, or about 11 KDa, about 12 KDa or about 13 KDa. In some embodiments, the size/molecular weight/molecular mass of the polypeptide in its trimeric form is from about 11 KDa to about 13 KDa, or about 11 KDa, about 12 KDa or about 13 KDa. In some embodiments, the size/molecular weight/molecular mass of the polypeptide or polypeptide oligomer or a part/fragment thereof is about 15 KDa, about 16 KDa, about 17 KDa, about 18 KDa, about 19 KDa or about 20 KDa.

In some embodiments, the polypeptide or the polypeptide in its monomeric form or a part/fragment thereof has a size/molecular weight/molecular mass of no more than about 6000 Da. In some embodiments, the polypeptide or the polypeptide in its monomeric form or a part/fragment thereof has a size/molecular weight/molecular mass of from about 5000 Da to about 6000 Da or from about 5200 Da to about 5600 Da. In various embodiments, the polypeptide or the polypeptide in its monomeric form or a part/fragment thereof has a size/molecular weight/molecular mass of about 5240 Da, about 5250 Da, about 5260 Da, about 5270 Da, about 5280 Da, about 5290 Da, about 5300 Da, about 5310 Da, about 5320 Da, about 5380 Da, about 5390 Da, about 5400 Da, about 5520 Da, about 5530 Da, about 5265 Da, about 5267 Da, about 5283 Da, about 5313 Da, about 5246 Da, about 5264.87 Da, about 5266.85 Da, about 5282.83 Da, about 5312.84 Da, about 5399.85 Da, about 5529.94 Da or about 5245.78 Da.

In some examples, the polypeptide (or a part/fragment thereof) acts as a ligand or a signaling molecule. In some examples, the polypeptide (or a part/fragment thereof) exerts its biological functions by engaging with and/or modulating one or more regulatory proteins, including but not limited to those larger than itself. In some examples, the polypeptide (or a part/fragment thereof) interacts with or binds to subunit 9 of mitochondrial respiratory complex III.

In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript is implicated in or capable of regulating cell division, cell proliferation and/or cell dedifferentiation of a heart cell.

In some examples, ATG-mutated embryonic stem (ES) cell clones
5 generated using CRISPR gene editing technique, which disrupts the formation of the polypeptide while still keeping its RNA transcript, fails to use fatty acid to generate adenosine triphosphate (ATP). In some examples therefore, the polypeptide (or a part/fragment thereof) allows for fatty acid utilization in a heart cell. In some examples, this capability is not demonstrated by the RNA transcript
10 of the polypeptide.

Mouse cardiomyocytes (CMs) exit cell cycle at the end of the proliferation window at approximately the seventh postnatal day (P7). In some examples, expression of the polypeptide (or a part/fragment thereof) and/or its RNA transcript transiently spikes at P7 and increases progressively from P10 onwards
15 with age in mouse hearts, indicating its potential role at regulating CM cell cycle exit during this stage of development.

In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript regulates cell proliferation by regulating cell cycle re-entry. In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA
20 transcript regulates cell cycle re-entry by regulating cell-cycle checkpoints regulators. In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript regulates one or more of the following cell-cycle checkpoints regulators: activators, inhibitors, G1/S and/or G2/M phase regulators.

In some examples, the polypeptide (or a part/fragment thereof) and/or its
25 RNA transcript regulates one or more of the following G1/S phase regulators: Cdk4, Cdk6, Ccne1 and Ccnd2. In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript regulates one or more of the following G1/S phase regulators: Cdk6 and Ccnd2. In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript regulates one
30 or more of the following G2/M phase regulators: Ccng1, Cdk1 and Cdc25a.

In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript regulates cyclin-dependent kinase inhibitor 1 (p21), which is a

G1/S and G2/M phase cell cycle inhibitor. In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript regulates p21 through regulating upstream Calreticulin (CALR). In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript regulates CALR to regulate cell
5 cycle re-entry.

In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript downregulates Ccnd2. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript upregulates
10 Ccng1. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript does not significantly change the expression of Nppa and/or Dstn.

In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript upregulates the
15 G1/S phase activator Cdk6. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript upregulates the G2/M phase activators Cdk1 and Cdc25a. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript induces or promotes S phase
20 entry. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript induces or promotes M phase entry. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript significantly increases Aurora B which is a marker for cytokinesis. In some
25 examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript induces or promotes cytokinesis.

In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript downregulates the G1/S and G2/M phase inhibitor p21 and/or upregulates CALR. In some
30 examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript upregulates CALR which blocks p21 translation. In some examples, the effects of a reduced/abolished

expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript is not recapitulated by loss of p21 alone.

In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript decreases a
5 cross-sectional area of a cell e.g. a CM cell or decreases a cell size.

Thus, in various examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript induces or promotes cell cycle re-entry *in vitro* and *in vivo*.

In some examples, a reduced/abolished expression, or inhibition of the
10 polypeptide (or a part/fragment thereof) and/or its RNA transcript does not significantly change the levels of one or more of the following G1/S phase activators: Ccne2, Ccnd1 and Cdk2. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript does not significantly change the levels of one or more of the
15 following G2/M phase activators: Ccnb1 and Cdc25b. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript does not significantly increase or induce apoptosis or cell death.

In some examples, a reduced/abolished expression, or inhibition of the
20 polypeptide (or a part/fragment thereof) and/or its RNA transcript increases the expression of a dedifferentiation marker such as DAB2. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript results in a dedifferentiated profile such as a DAB2+ profile.

25 In various examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript, e.g. in a heart cell, leads to the heart cell acquiring cardiac progenitor-like characteristics (e.g. cell appears rounder, smaller, and/or with higher nucleus/cytoplasm ratio compared to a wild-type cardiomyocyte, and/or cell shows increased DAB2+
30 profile and/or there is a loss of tissue structure).

Thus, in various examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript, e.g. in a heart cell, induces or promotes dedifferentiation e.g. of the heart cell.

In some examples, the polypeptide (or a part/fragment thereof) and/or its
5 RNA transcript is upregulated in a Transverse Aortic Constriction (TAC) stress mouse model. In some examples, the polypeptide (or a part/fragment thereof) and/or its RNA transcript is upregulated in diseased hearts such as those of end-stage heart failure patients suffering from dilated cardiomyopathy, hypertrophic cardiomyopathy and/or ischaemic cardiomyopathy.

10 In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript results in a partial and significant rescue of ejection fraction (EF%) and left ventricular (LV) wall thickness in a TAC stress mouse model. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment
15 thereof) and/or its RNA transcript in a TAC stress mouse model induces dedifferentiation of heart cells. In some examples, a reduced/abolished expression, or inhibition of the polypeptide (or a part/fragment thereof) and/or its RNA transcript in a TAC stress mouse model leads to more DAB2+ cardiomyocytes (which may be smaller and predominantly mononucleated as
20 compared to DAB2- cardiomyocytes) per heart section as compared to a control mouse.

Accordingly, it may be desirable to manipulate the polypeptide (or a part/fragment thereof) to disrupt or inhibit its native functions to achieve heart cell proliferation or dedifferentiation. For example, one or more mutations may be
25 introduced in the polypeptide (or a part/fragment thereof) to partially or wholly inactivate its native functions. For example, one or more of a loss-of-function mutation, a null mutation, an inactivating mutation, a recessive mutation and a dominant negative mutation may be introduced in the polypeptide (or a part/fragment thereof). In various embodiments therefore, there is provided the
30 polypeptide (or a part/fragment thereof) as described herein comprising one or more mutation(s).

Further, the native biological effects exerted by the polypeptide (or a part/fragment thereof) may also be blocked or inhibited through use of an agent. In various embodiments, there is provided an agent that is capable of interacting with the polypeptide (or a part/fragment thereof); capable of interfering with or blocking an interaction of the polypeptide (or a part/fragment thereof) with biomolecule(s) such as a cell-cycle checkpoints regulator(s) and/or CALR; capable of interfering with or blocking an oligomerisation of the polypeptide (or a part/fragment thereof); capable of inhibiting/blocking a translation of a RNA to produce the polypeptide (or a part/fragment thereof); and/or capable of interacting with or inhibiting a polynucleotide encoding for the polypeptide (or a part/fragment thereof); so to regulate cell division, cell proliferation and/or cell dedifferentiation of a heart cell. In various embodiments, said interacting with the polypeptide (or a part/fragment thereof) comprises binding at least partially to the polypeptide (or a part/fragment thereof) and/or at least partially cleaving/degrading/digesting/hydrolyzing the polypeptide (or a part/fragment thereof) such that the polypeptide (or a part/fragment thereof) no longer has biological activity or has reduced biological activity.

In various embodiments, the agent comprises an inhibitor targeted at the polypeptide (or a part/fragment thereof). In various embodiments, there is provided an inhibitor of the polypeptide (or a part/fragment thereof). In various embodiments, the agent or the inhibitor comprises a pharmaceutically effective compound, a drug, a small molecule, a nanoparticle, a peptide, a peptide corresponding substantially to the polypeptide (or a part/fragment thereof) and further comprising mutation(s), a protein, a protease, a nucleic acid, an antibody and fragments e.g. an antigen-binding fragment and combinations thereof.

Methods of identifying an agent or inhibitor having the desired biological activity are known to the skilled person. For example, screening or high-throughout screening of compound libraries may be employed to identify lead compounds that display desirable biological activity e.g. binding affinity for the polypeptide (or a part/fragment thereof) and/or inhibitory/antagonistic activity against the polypeptide (or a part/fragment thereof). Cell-based screening or cell-based assays, e.g. using a heart cell or human ES-derived CMs or Engineering

Heart Tissue (EHT) system, may be used. Multicellular, co-culture, 3D spheroid, or more specifically heart tissue and heart organoid may also be used as platforms for drug discovery. Other routine techniques such as rational design, substrate or ligand modelling and fragment screening may also be employed in combination, or independently. Antibodies may also be generated by immunizing animals with the polypeptide (or a part/fragment thereof) and engineering the antibodies so obtained to prevent an unintended immune response in the subject e.g. a human subject through strategies such as chimerization or replacement of the constant regions of the animal antibodies with those of a human antibody etc.

10 In various embodiments, the agent or inhibitor is capable of penetrating, translocating to or being taken up by a mitochondria, optionally wherein the agent or inhibitor is capable of selectively penetrating, translocating to or being taken up by a mitochondria, further optionally wherein the agent or inhibitor comprises a mitochondrial targeting signal, optionally a mitochondrial membrane targeting signal. In various embodiments, the agent or inhibitor is coupled/conjugated to a carrier capable of penetrating, translocating to or being taken up by a mitochondria, optionally wherein the carrier is capable of selectively penetrating, translocating to or being taken up by a mitochondria. For example, carriers that may penetrate the mitochondria include short peptide sequences with alternating cationic and hydrophobic residues, oligomeric carbohydrate scaffold, vesicle-based transporter system, cationic liposome etc.

In various embodiments, there is provided the agent or the inhibitor for use in therapy. In various embodiments, there is provided the agent or the inhibitor for use in treating impaired heart function. In various embodiments, there is provided use of the agent or the inhibitor or its pharmaceutically acceptable salt/derivatives thereof in the manufacture of a medicament for the treatment of impaired heart function.

25 In various embodiments, there is provided a composition, optionally a pharmaceutical composition, comprising the inhibitor or the agent and a suitable carrier, adjuvant, diluent and/or excipient.

30 In various embodiments, the agent, the inhibitor or the composition is capable of at least partially rescuing or improving heart function. In various

embodiments, the agent, the inhibitor or the composition is capable of at least partially reducing or improving heart function after onset of hypertrophy. In various embodiments, said rescuing or improving heart function comprises at least partially rescuing or improving one or more of the following selected from
5 the group consisting of: ejection fraction, left ventricle wall thickness, right ventricle wall thickness, left ventricular wall stress, right ventricular wall stress, ventricular mass, contractile function, cardiac hypertrophy, end diastolic volume, end systolic volume, cardiac output, cardiac index, pulmonary capillary wedge pressure and pulmonary artery pressure. In various embodiments, the agent, the
10 inhibitor or the composition is capable of stimulating cardiomyocyte repair. In various embodiments, the agent, the inhibitor or the composition is capable of stimulating cardiac regeneration and/or proliferation.

In various embodiments, there is provided the agent, the inhibitor or the composition for use as a cardiac regenerative agent, a cardiac proliferative agent
15 and/or a cardiac protective agent.

In various embodiments, the agent, the inhibitor or the composition is capable of inducing proliferation of heart cell in the subject. In various embodiments, the agent, the inhibitor or the composition is capable of inducing dedifferentiation of heart cell in the subject.

20 In various embodiments, the agent, the inhibitor or the composition comprises a first agent, inhibitor or composition targeted at the polypeptide (or a part/fragment thereof) and a second agent, inhibitor or composition targeted at the polynucleotide encoding for the polypeptide (or a part/fragment thereof).

In various embodiments, there is provided a method of treating impaired
25 heart function in a subject, the method comprising: administering the agent, the inhibitor or the composition to the subject. In various embodiments, there is provided a method of treating impaired heart function in a subject, the method comprising: inhibiting the polypeptide (or a part/fragment thereof) in the subject.

In various embodiments, there is provided a method of treating or
30 preventing a heart disorder or impaired heart function in a subject in need thereof, the method comprising reducing/suppressing/blocking/inhibiting/downregulating/modulating an expression of the polypeptide (or a part/fragment thereof) or an

expression of a biomolecule downstream or upstream of the polypeptide (or a part/fragment thereof). In various embodiments, there is provided a method of treating or preventing a heart disorder or impaired heart function in a subject in need thereof, the method comprising administering to said subject a
5 therapeutically effective amount of an agent or an inhibitor that is capable of binding to or interacting with the polypeptide (or a part/fragment thereof) to reduce/suppress/block/inhibit/downregulate/modulate an expression of the polypeptide (or a part/fragment thereof) or an expression of a biomolecule downstream or upstream of the polypeptide (or a part/fragment thereof). In
10 various embodiments, there is provided a method of treating or preventing a heart disorder or impaired heart function in a subject in need thereof, the method comprising reducing/suppressing/blocking/inhibiting an oligomerisation of the polypeptide (or a part/fragment thereof). In some embodiments, reducing/suppressing/blocking/inhibiting/downregulating/modulating an
15 expression (e.g. a functional expression) of the polypeptide (or a part/fragment thereof) comprises reducing/suppressing/blocking/inhibiting an oligomerisation of the polypeptide (or a part/fragment thereof).

In various embodiments, the heart disorder or the impaired heart function is selected from the group consisting of: myocardial infarction, heart failure,
20 coronary artery disease, narrowing of the arteries, heart attack, abnormal heart rhythms, arrhythmias, heart failure, heart valve disease, congenital heart disease, heart muscle disease, cardiomyopathy (including dilated cardiomyopathy, hypertrophic cardiomyopathy, ischaemic cardiomyopathy and idiopathic cardiomyopathy), pericardial disease, aorta disease, marfan syndrome, genetic
25 cardiomyopathy, non-genetic cardiomyopathy, heart hypertrophy, pressure overload-induced heart dysfunction, and damaged heart tissue.

In various embodiments, there is provided a method of assessing heart function in a subject, the method comprising: determining an expression level of the polypeptide (or a part/fragment thereof) in a sample obtained from the subject;
30 comparing the expression level to a reference expression level, wherein if the expression level deviates from or exceeds the reference expression level, the subject is considered to have impaired or deteriorated heart function. In some

embodiments, if the expression level in the sample deviates from or exceeds the reference expression level, it is indicative of a heart disorder/impaired heart function or a predisposition to develop a heart disorder/impaired heart function in the subject.

5 In some embodiments, the reference expression level comprises an expression level of the polypeptide (or a part/fragment thereof) in a healthy population. For example, the reference expression level may be the average expression level/range of the polypeptide (or a part/fragment thereof) in a healthy population. In some embodiments, the reference expression level comprises an
10 expression level of the polypeptide (or a part/fragment thereof) in an earlier sample obtained from the subject.

 In various embodiments, there is provided a method of monitoring heart function in a subject, the method comprising: determining a first expression level of the polypeptide (or a part/fragment thereof) in a first sample obtained from the
15 subject at a first time point; determining a second expression level of the polypeptide (or a part/fragment thereof) in a second sample obtained from the subject at a second time point occurring after the first time point; and comparing the first expression level with the second expression level, wherein if the second expression level is higher than the first expression level, then the heart function
20 is considered to have deteriorated in the subject, wherein if the second expression level is lower than the first expression level, then the heart function is considered to have improved in the subject, and wherein if the second expression level is substantially the same as the first expression level, then the heart function is considered to have stabilised in the subject. In some embodiments, if the
25 second expression level is lower than the first expression level, it is indicative of a favourable prognosis of the subject.

 In various embodiments, determining an expression level of the polypeptide (or a part/fragment thereof) comprises assaying for the concentration and/or functional activity of said polypeptide (or a part/fragment thereof),
30 including use of a competitive binding assay for said polypeptide (or a part/fragment thereof).

In various embodiments, determining an expression level of the polypeptide (or a part/fragment thereof) comprises contacting the sample with a reagent specific to the polypeptide for ascertaining or measuring quantitatively, semi-quantitatively or qualitatively the amount of polypeptide (or a part/fragment thereof). The agent may be capable of detecting and/or binding directly or indirectly to the polypeptide (or a part/fragment thereof). Examples of reagents include but are not limited to proteins/probes (for example antigen binding proteins such as antibodies or fragments thereof, enzymes such as horseradish peroxidases and alkaline phosphatase, and the like), polynucleotides (for example aptamers), and small molecules (for example metallic nanoparticles). An expression level of the polypeptide (or a part/fragment thereof) may be determined by a number of routine techniques including immunohistochemistry, ELISA, Western blot, dot blot, immunoprecipitation, mass spectrometry and the like. For example, a reagent such as an antibody (e.g. a labeled antibody) that specifically binds the polypeptide (or a part/fragment thereof) may be added to the sample to permit a relative or absolute ascertaining of the amount of polypeptide (or a part/fragment thereof).

The sample may comprise a heart tissue, optionally a mitochondrion from a heart tissue. In various embodiments therefore, the method further comprises extracting a mitochondrion from a heart tissue. In various embodiments, the heart tissue comprises a left ventricular heart tissue. In various embodiments, the heart tissue comprises a cardiomyocyte.

In various embodiments, the subject comprises a human subject.

In some embodiments, the method comprises a prognosis method. In some embodiments, the method comprises a diagnosis method.

In various embodiments, there is provided a prognostic or diagnostic method to assess the regenerative or proliferative capacity of heart tissue before, after or during a cardiac treatment regimen comprising: determining the presence or amount of the polypeptide (or a part/fragment thereof) in a cardiac sample of said heart tissue; and where the polypeptide (or a part/fragment thereof) is present concluding the proliferative capacity of said heart tissue is poor; and where the polypeptide (or a part/fragment thereof) is absent concluding the

proliferative capacity of said heart tissue is good. In some embodiments, the determining step involves assaying for the functional activity of said polypeptide (or a part/fragment thereof), including use of a competitive binding assay for said polypeptide (or a part/fragment thereof).

5 In various embodiments, the method further comprises administering to the subject an inhibitor of the polypeptide (or a part/fragment thereof). In various embodiments, the method further comprises administering to the subject an agent, an inhibitor or a composition as described herein. In various embodiments, the method further comprises administering to the subject a therapeutically
10 effective amount of the inhibitor, the agent, or the composition.

In various embodiments, there is provided a method of identifying or screening for a potential drug or therapeutic agent for treating impaired heart function or heart disorder, the method comprising: analyzing an expression level of the polypeptide (or a part/fragment thereof) in the presence and in the absence
15 of the candidate drug or therapeutic agent; and determining if the candidate drug or therapeutic agent is a useful drug or therapeutic agent for treating or preventing an impaired heart function or heart disorder based on the differences in the expression of the polypeptide (or a part/fragment thereof) in the presence of the candidate drug or therapeutic agent and in the absence of the candidate drug or
20 therapeutic agent.

In various embodiments, the candidate drug or therapeutic agent is identified as a useful drug or therapeutic agent for treating or preventing an impaired heart function or a heart disorder if the expression of the polypeptide (or a part/fragment thereof) is reduced in the presence of the candidate drug or
25 therapeutic agent as compared to in the absence of the candidate drug or therapeutic agent.

In various embodiments, there is provided a method of identifying a potential drug for treating impaired heart function, the method comprising: determining a first expression level of the polypeptide (or a part/fragment thereof)
30 in a cell; exposing the cell to a drug candidate; and determining a second expression level of the polypeptide (or a part/fragment thereof) after the exposure, wherein if the second expression level is lower than the first expression

level, then the drug candidate is identified as a potential drug for treating impaired heart function.

In various embodiments, there is provided a vector comprising a polynucleotide sequence e.g. a DNA/cDNA sequence encoding for the polypeptide (or a part/fragment thereof) or the agent, the inhibitor or the composition as described herein. The vector may be selected from the group consisting of a plasmid, a viral particle, a phage, a baculovirus, a yeast plasmid, a lipid based vehicle, a polymer microsphere, a liposome, and a cell based vehicle, a colloidal gold particle, lipopolysaccharide, polypeptide (or a part/fragment thereof), polysaccharide, a viral vehicle, an adenovirus, a retrovirus, a lentivirus, an adeno-associated viruses, a herpesvirus, a vaccinia virus, a foamy virus, a cytomegalovirus, a Semliki forest virus, a poxvirus, a pseudorabies virus, an RNA virus vector, a DNA virus vector and a vector derived from a combination of a plasmid and a phage DNA. The polynucleotide may be operatively linked to an expression control sequence(s) to direct peptide synthesis. The vector may also comprise one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells.

In various embodiments, there is provided a host cell transfected with or comprising the vector.

In various embodiments, there is provided a method for producing the polypeptide (or a part/fragment thereof), the agent, the inhibitor or the composition, the method comprising: culturing the host cell under suitable conditions to permit production of the polypeptide (or a part/fragment thereof), the agent, the inhibitor or the composition; and recovering the polypeptide (or a part/fragment thereof), the agent, the inhibitor or the composition so produced.

The vector and/or the host cell may be used in a method of therapy.

In various embodiments, there is provided a transgenic non-human subject comprising a nucleic acid construct or a polynucleotide construct (e.g. DNA/cDNA sequences) encoding for the polypeptide (or a part/fragment thereof), optionally wherein the transgenic non-human subject overexpresses the polypeptide (or a part/fragment thereof). The transgenic non-human subject may

comprise a non-human mammal selected from the group consisting of a non-human primate, a rodent, a mouse, a rabbit, a sheep, a cow and a pig.

In various embodiments, there is provided the polypeptide (or a part/fragment thereof) coupled to a detectable label. The polypeptide (or a
5 part/fragment thereof) may act as a biomarker for identifying impaired heart function.

In various embodiments, there is provided a kit comprising the detectable label or the agent. The detectable label may be selected from the group consisting of fluorescent label, radioactive label, enzymatic label,
10 chemiluminescent label, oligonucleotide, dye, colloidal particle, oxidant, reductant and combinations thereof. The kit may be a kit for assessing heart function in a subject, or for assessing whether a subject has a heart disorder or is predisposed to developing a heart disorder. The kit may further comprise instructions for use.

15 In various embodiments, there is provided a method for proliferation, dedifferentiation or regeneration, or promoting proliferation, dedifferentiation or regeneration of a heart cell, the method comprising contacting the heart cell with one or more of the agent, the inhibitor, the composition or the vector. In various embodiments, there is provided a method of dedifferentiating and/or proliferating
20 a heart cell, the method comprising: inhibiting the expression of the polypeptide (or a part/fragment thereof) in the heart cell.

In various embodiments, there is provided a cell, optionally a heart cell, produced by the method, or progenies or cell derivatives thereof. In various
25 embodiments, there is provided the heart cell, or progenies or cell derivatives thereof, for use in therapy in a subject in need thereof.

In various embodiments, the heart cell comprises a cardiomyocyte, further optionally wherein the cardiomyocyte comprises an adult cardiomyocyte.

In various embodiments, the method described herein is *in vitro* or *in vivo*.

In various embodiments, there is provided a product, a method or a use
30 as described herein.

BRIEF DESCRIPTION OF FIGURES

FIG. 1 is a mass spectrum result of the membrane proteins extracted from the mitochondria of a rat heart tissue.

5 FIG. 2 is a deconvolution of the mass spectrum of FIG. 1.

FIG. 3 is a sequencing result of the peptide, with a peak at 886.48 m/z, using tandem mass spectrometry (ms/ms).

FIG. 4. Peptide mapping and coverage of STMP1 in mammalian mitochondria samples.

10 FIG. 5. Preliminary evidence indicates SGHRT micropeptide oligomerisation. A, 1ug and 3ug of SGHRT synthesised peptides were boiled for 10 mins and run in 16.5% Tricine gel and the Gel was stained with Coomassie Blue solution. B, 3ug of SGHRT synthesised peptides were treated with 1mM and 10 mM EDTA for 30 mins and then run in 16.5% Tricine gel and the Gel was stained with Coomassie Blue solution. C, Analytical Ultracentrifugation (AUC) analysis of SGHRT synthesised peptides suggested that molecular weight of SGHRT peptides is in the range of 15-20 KDa, and the highest peak is at ~17 KDa. D, Western blot with FLAG antibody showing a 12 KDa band in protein lysates of HEK cells transfected with SGHRT-FLAG vector in which SGHRT was C-terminal FLAG tagged, but not in lysates from cells transfected with FLAG-SGHRT vector in which SGHRT was N-terminal FLAG-tagged or with ATG-mutated SGHRT-FLAG.

15 FIG. 6. Upregulation of SGHRT in human hearts with End-stage HF. A, RNA-seq tracks showing higher expression of SGHRT in Dilated Cardiomyopathy (DCM), Hypertrophic cardiomyopathy (HCM) and Ischemic Cardiomyopathy (IHD) patients compared to healthy hearts. B, RT-qPCR validation of SGHRT upregulation in cardiac tissue from patients with end-stage HF.

20 FIG. 7. SGHRT knockout in hESC-CMs results in increased DAB2+ CMs. A, RNA-seq data showing increased mRNA expression of DAB2 in D58 SGHRT KO hESC-CMs. B, Western blot result showing increased DAB2 protein expression in D58 SGHRT KO hESC-CMs. GAPDH was used as loading control.

25 FIG. 8. SGHRT knockout in hESC-CMs results in increased DAB2+ CMs. A, RNA-seq data showing increased mRNA expression of DAB2 in D58 SGHRT KO hESC-CMs. B, Western blot result showing increased DAB2 protein expression in D58 SGHRT KO hESC-CMs. GAPDH was used as loading control.

30 FIG. 9. SGHRT knockout in hESC-CMs results in increased DAB2+ CMs. A, RNA-seq data showing increased mRNA expression of DAB2 in D58 SGHRT KO hESC-CMs. B, Western blot result showing increased DAB2 protein expression in D58 SGHRT KO hESC-CMs. GAPDH was used as loading control.

C, Representative microscope images showing an increase in DAB2+ hESC-CMs and a decrease in cTnT+ hESC-CMs after SGHRT knockout at D58. White boxed areas are magnified to 40X. D, SGHRT knockout resulted in increased DAB2+ CMs at D58 time points.

5 FIG. 8. SGHRT KO EHT show poorly formed EHT profile and increased dedifferentiated DAB2+ CMs. A, Representative pictures of EHT generated from wildtype and SGHRT knockout hESC-CMs at D16. B, Contractility profile of SGHRT WT and KO of D16 EHT. C, Representative microscope images showing an increase in DAB2+ CMs in SGHRT KO D14 EHT.

10 FIG. 9. Sghrt knockdown resulted in CM dedifferentiation in neonatal (P7) mouse. A, Expression of Sghrt in whole hearts from P1 to P56 mice. B, Injection of AAV9-cTNT-GFP-RNAi to knockdown Sghrt in P7 WT mice. C, Representative pictures of a typical DAB2+ and cTNI+ cardiomyocytes, confocal images with z-stacking showing co-localization of DAB2 and cTNI signals. D, Quantification of
15 DAB2+ CMs per heart section from control mice and mice with Sghrt KD (2 sections from each heart were analysed). E, Cell sizes of DAB2+ CMs and their neighbouring DAB2- CMs. F, Representative pictures of a binucleated DAB2+ CM (left, the two nuclei are indicated by the white arrows) and a mononucleated DAB2+ CM (right). G, Graph showing the percentage of binucleated and
20 mononucleated DAB2+ CMs. ****P < 0.0001 (Student's t-test).

 FIG. 10. KD of Sghrt rescued cardiac function and induced CM dedifferentiation in TAC mouse model within 6 weeks. A, Single nuclear RNA-seq show an upregulation of Sghrt mRNA expression in single nucleus isolated from
25 TAC CMs compared to those from Sham CMs. Each dot represents a single CM nucleus. B, Diagram showing the timeline of TAC surgery on 8-week-old WT mice, followed by AAV9 injection and harvesting. C, Graphs showing EF and FS of Sham mice, control mice with TAC and Sghrt KD mice with TAC, 6 weeks after AAV9 injection. D, Representative pictures of a typical DAB2+ and cTNI+ cell. E, Quantification of DAB2+ CMs per heart section (3 sections from each heart were
30 analysed). F, The cell size of DAB2+ CMs and their neighbouring DAB2- CMs. G, Representative pictures of a binucleated DAB2+ CM (top panel) and a mononucleated pH3+ CM (bottom panel). (I) Graph showing the percentage of

binucleated and mononucleated DAB2+ CMs. n.s. $P > 0.05$, ** $P < 0.01$, **** $P < 0.0001$ (Student's t-test).

FIG. 11. A, Representative DNA sequencing result confirmed that 2 A-to-G (BE19-4 and BE19-10) and 2 C-to-T (BE20-1 and BE20-2) based editing ES clones were successfully generated. B, Q-PCR results showed no change in SGHRT RNA expression in BE ES clones. C, Quantification of ATP production in WT, KO and BE ES-derived CM clones with and without Fatty Acid treatment.

EXAMPLES

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Example embodiments of the disclosure will be better understood and readily apparent to one of ordinary skill in the art from the following discussions and if applicable, in conjunction with the figures. It should be appreciated that other modifications related to structural, electrical and optical changes may be made without deviating from the scope of the invention. Example embodiments are not necessarily mutually exclusive as some may be combined with one or more embodiments to form new exemplary embodiments.

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Key regulators of the cardiomyocytes dedifferentiation and proliferation

The neonatal mammalian heart possesses a robust but transient regenerative ability that is rapidly lost after the first few days of life. The cardiac regeneration is likely due to dedifferentiation and subsequent proliferation of pre-existing CMs. Dedifferentiation of cardiomyocytes is characterized by the disassembly of sarcomeric structure, extrusion of mitochondria, electrical uncoupling and expression of regulators of cell cycle progression. Understanding the biology of CM dedifferentiation and proliferation could be of great clinical significance for treating heart failure.

Signaling pathways, transcription factors, cell cycle regulators, epigenetic modifiers, environmental factors and extracellular matrix have been identified to control mammalian CM dedifferentiation and proliferation. Manipulating these pathways reactivates CM proliferation and regenerates the injured adult

mammalian hearts. In human HF, the limited functional recovery clearly demonstrates insufficient regeneration of human adult CMs. The evidence of human CM renewal suggests that the development of pharmacological strategies to stimulate this process may be a rational alternative or complement to cell transplantation strategies for CM replacement. Therefore, it is important to understand the biology and uncover novel gene regulatory pathways/drivers mediating CM dedifferentiation and proliferation in normal and failing hearts.

Long non-coding RNA-derived micropeptides in cardiomyopathy

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Long noncoding RNAs (lncRNAs) has been recently identified as a new layer of complexity to the regulation of the biological processes underlying normal cardiac development and myocardial remodeling during disease. Dysregulation in lncRNA regulatory circuits have been associated with cardiac pathological hypertrophy and heart failure. LncRNAs represent potential targets for novel therapeutic strategies for cardiovascular diseases. Interestingly, recent emerging evidence indicates that several LncRNAs have been mis-annotated as noncoding and in fact contains short open reading frames (sORFs) that encode functional peptides. sORF-encoded peptides (SEPs), or micropeptides, have been shown to have important roles in fundamental biological processes and in the maintenance of cellular homeostasis. These small proteins can act independently as ligands or signaling molecules, or they can exert their biological functions by engaging with and modulating larger regulatory proteins. Biological roles have been discovered to a small fraction of the total putative micropeptides and a huge amount of work remains to be done to prove their existence and elucidate their functions.

Single nuclear RNA-seq was previously performed on left ventricular (LV) CMs isolated from healthy and failing adult mouse and human hearts. This was followed by a mapping out of gene regulatory networks that contained key nodal lincRNAs (Sghrt and Gas5) that regulate dedifferentiation and cell cycle gene expression in CM subpopulations. This was also described in International

Application No. PCT/SG2017/050620, the contents of which are fully incorporated herein by reference.

Mass spectrometry to profile small peptides in mitochondrial extract of bovine hearts identified that Sghrt is also potentially a micropeptide that has 100% homology with "Short transmembrane mitochondrial protein 1 precursor" (STMP1) and was co-purified with subunit 9 of mitochondrial respiratory complex III. Multiple sequence alignment demonstrated that the STMP1 micropeptide sequence is also highly conserved across vertebrates. Because there is no experimental evidence yet confirming the presence of Sghrt micropeptide in human and other mammals, it was proposed to perform Mass Spectrometry analysis on mitochondrial membrane proteins isolated from mouse, rat, pig and human heart as well as human pluripotent stem cell-derived cardiomyocytes.

Identification Sghrt-encoded micropeptides in mitochondria membrane proteins isolated from rat hearts

Predicted mass-to-charge ratios (m/z) of peptides of interest were generated using an online tool (<http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msisotope>) (see sequences in Zhang D et al., Functional prediction and physiological characterization of a novel short trans-membrane protein 1 as a subunit of mitochondrial respiratory complexes. *Physiol Genomics*. 2012; 44: 1133–1140. doi: 10.1152/physiolgenomics.00079.2012.). The m/z ratios allow for identification of the right peak of mass in a mass spectrum and consequently, the identification of the peptide sequence of that peak. The results are shown in Table 2 below.

Table 2: Predicted mass and m/z of the membrane peptide SGHRT in various animal species

Protein	Amino acid sequence	Score	77%	78%	79%	80%
Human PTPN22 (human)	MIQLFLGTLGNYVGMVLAQNYDIENLAKKLESDINKKLEAKKPPEN	5264.87	753.13	878.48	1053.98	1
PTN (mouse) (mouse)	conserved					
Mouse PTPN22 (mouse)	conserved					
Human PTPN22	MIQLFLGTLGNYVGMVLAQNYDIENLAKKLESDINKKLEAKKPPEN	5266.85	753.41	878.82	1054.38	2
Human PTPN22	MIQLFLGTLGNYVGMVLAQNYDIENLAKKLESDINKKLEAKKPPEN	5267.83	755.70	881.48	1057.37	3
Human PTPN22	MIQLFLGTLGNYVGMVLAQNYDIENLAKKLESDINKKLEAKKPPEN	5268.82	759.38	886.48	1063.57	4
Human PTPN22	MIQLFLGTLGNYVGMVLAQNYDIENLAKKLESDINKKLEAKKPPEN	5269.89	772.41	900.98	1080.98	5
Human PTPN22	MIQLFLGTLGNYVGMVLAQNYDIENLAKKLESDINKKLEAKKPPEN	5529.94	793.03	922.66	1156.99	6
Human PTPN22	MIQLFLGTLGNYVGMVLAQNYDIENLAKKLESDINKKLEAKKPPEN	5245.78	750.40	875.30	1050.16	7

Mitochondrial membrane proteins were extracted from rat heart tissue and subjected to liquid chromatography–mass spectrometry (LC-MS). Presence of the predicted m/z was determined by using the extracted ion chromatogram (EIC) function of Agilent Mass Hunter B08 software. The mass spectrum obtained is shown in FIG. 1. A prominent molecular feature 886.48 m/z eluting at around 9 minutes was identified.

The mass spectrum was then deconvoluted to obtain estimates of original molecular mass (FIG. 2). The prominent molecular feature 886.48 m/z was estimated to be of mass 5321.84, which is suggestive of the rat STMP1 peptide.

For confirmation of peptide identity, amino acid sequencing was done using tandem mass spectrometry (ms/ms). The sample was rerun on the LC-MS, with the collision-induced dissociation (CID) cell configured to fragment the molecular feature at various fragmentation energies, and the resulting mass spectrum was collected. Amino acid sequences were then confirmed by manually matching the ms/ms spectrum to predicted a, b, and y-series ions obtained from <http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msproduct> (FIG. 3). Several different fragments were identified, including for example, a fragment comprising the sequence "MLQFLLGFTL". Based on the identified fragments, the full sequence of Sghrt is predicted to be "MLQFLLGFTLGNVVGMYLAQNYDIPNLAKKLEEIKKDLDAKKKPPSA". The ms/ms spectrum is in agreement with b ions generated from the N-terminus of rat Sghrt (see predicted N-terminal amino acids of rat Sghrt in Table 2). Strong signal of a2 and y4 ions were also noted.

25 Mass spectrometry analysis was performed in a further experiment to confirm and resolve the protein sequence for SGHRT in mouse, rat, pig and human. Samples were prepared for proteomics analysis using a gel-assisted sample preparation (GASP) strategy (Fischer, R. & Kessler, B. M. Gel-aided

sample preparation (GASP)--a simplified method for gel-assisted proteomic sample generation from protein extracts and intact cells. *Proteomics* 15, 1224-1229, doi:10.1002/pmic.201400436 (2015)). Peptide mapping was done in mitochondria enriched heart samples and hES-derived CM. To increase protein coverage, LC-MS/MS was done in undigested, trypsin digested, as well as chymotrypsin digested samples. Fragments of human SGHRT peptides were detected and confirmed in limited human heart cadaver samples available, and full coverage was achieved in rat and mouse samples (FIG. 4).

Sghrt locus is therefore found to harbour a sORF and encode for a 47-amino acid mitochondrial micropeptide (derived from a mitochondrial long noncoding RNA) that may account for Sghrt therapeutic and biomarker potential/value. The micropeptide is named Short transmembrane mitochondrial protein 1 precursor, or STMP1. A peptide sequence with 100% homology has been isolated in a proteome screen of bovine cardiac mitochondrial fractions, and co-purified with subunit 9 of mitochondrial respiratory complex III. Moreover, multiple sequence alignment demonstrates that the STMP1 micropeptide sequence is highly conserved across vertebrates and targeting this sequence by morpholinos in zebrafish produced a phenotype including cardiac edema. In the present disclosure, Sghrt peptide sequence and fragments thereof were identified from the heart tissue extracts of mouse, rat, pig and humans. The results showed that Sghrt peptide sequence is highly conserved among different species, including among mammalian species.

Evidence indicates oligomerization of SGHRT micropeptides

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The expected size of SGHRT micropeptide is ~5.8 KDa. However, when SGHRT synthesized peptides were run in 16.5% Tricine SDS gel, three bands were observed at ~5.8KDa, ~8 KDa and ~12 KDa, with the 12 KDa band being most abundant (FIG. 5A). This result suggests that SGHRT presents in an oligomer, which could be a dimer or trimer. The SGHRT oligomer was also resistant to heat (FIG. 5A) and EDTA treatment (FIG. 5B), suggesting that a covalent bond is not responsible for the oligomerisation. To analyze the molecular

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weight of SGHRT peptides quantitatively in solution, Analytical Ultracentrifugation (AUC) analysis of SGHRT synthetic peptides was performed. The result indicated that the molecular weight of SGHRT peptide is in the range of 15-20 KDa and the highest peak was again at ~17 KDa (FIG. 5C). To validate that SGHRT oligomerisation also occurs in vivo, HEK cells were transfected with SGHRT-FLAG or FLAG-SGHRT vectors in which SGHRT was FLAG-tagged at the C- or the N-terminus, respectively. Interestingly, a strong band was consistently identified at ~12 KDa when SGHRT-FLAG was C-terminal tagged, but not N-terminal. As additional control, this band was absent in HEK cells transfected with mutated SGHRT in which the ATG was replaced by GGG (FIG. 5D). Taken together, the results were consistent with the conclusion that SGHRT micropeptides form oligomers, which could be dimers or trimers.

SGHRT mRNA expression is upregulated in the left ventricles of end-stage HF patients

Human LV samples harvested with an ongoing protocol approved by the Papworth (Cambridge) Hospital Tissue Bank Review Board and the Cambridgeshire Research Ethics Committee (UK) were available. These samples were from patients undergoing cardiac transplantation for end-stage ischemic and idiopathic cardiomyopathy. Based on >50 of these samples, and a continuous constant supply every month, a genome-wide mapping of differential DNA methylation and H3K36me3 enrichment profiles, a landscape of DNA repeats and circular RNAs in human end-stage HF was done. Relevant to this Gap proposal, RNA-seq and RT-qPCR validation using the human LV samples showed that SGHRT mRNA levels were upregulated in a range of diseased HF hearts (FIG. 6, DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; IHD: ischaemic cardiomyopathy).

Increased dedifferentiated CMs in SGHRT-KO hES differentiation and Engineered Heart Tissue (EHT)

To validate whether *SGHRT* regulates differentiation of human CM, a *SGHRT* knockout hES cell-line was generated using CRISPR/CAS9 technology to specifically delete the promoter and first exon of the *SGHRT* gene. The efficient and precise genome edited in *SGHRT*-KO hESCs, with a complete loss of *SGHRT* mRNA expression, was validated. By RT-qPCR, immunostaining, and Western blot, it was proven that there was no change to OCT4 mRNA/protein and NANOG mRNA, showing that *SGHRT*-KO hESCs maintained their pluripotency, and stemness was not affected by *SGHRT* KO.

Next, CM differentiation was performed using *SGHRT*-KO hESCs and harvested CM cultures at D58. *SGHRT*-KO CMs (MYH6-GFP reporter positive) appeared rounder, smaller, and with higher nucleus/cytoplasm ratio compared to the WT CMs (data not shown), suggesting that these cells may be acquiring cardiac progenitor-like characteristics. DAB2 mRNA and protein increase was also proven with RNA-seq and Western blot (FIG. 7A-B). There was significant increase in DAB2⁺ cells in *SGHRT*-KO (<1% in the WT compared to 11.2% DAB2⁺ CMs in D58 *SGHRT* KO, Fig. 1C-D). Consistently, there was also a significant decrease in cTnT⁺ (96.0% in WT cultures compared to 71.3% in D58 *SGHRT*-KO). These results lend further suggestion that dedifferentiated CMs lose CM characteristics and take on more progenitor-like characteristics. Moreover, using an EHT system that the lab has now robustly established (<http://foo-lab.com/video/EHT.html>) in collaboration with Thomas Eschenhagen (Hamburg), constructed EHT using WT and KO D14 hES-CM showed a loss in tissue structure (FIG. 8A-B) and increased DAB2⁺ CMs (FIG. 8C). Taken all together, the results thus far showed that the loss of *SGHRT* increases dedifferentiation of mature CMs both *in vivo* and *in vitro*.

Sghrt KD induces dedifferentiation of neonatal mouse cardiomyocytes *in vivo*

Mouse CMs enter cell cycle arrest at the end of their proliferation window at the 7th postnatal day (P7). Hence, *Sghrt* transcript levels during normal mouse heart development across this proliferation time-course window were assessed. It was found that *Sghrt* expression increased progressively from P10 onwards

with age (FIG. 9A). The end of the proliferation window at P7 coincided with a small but statistically significant expression spike in *Sghrt*. To test if *Sghrt* regulates CM dedifferentiation *in vivo* and assess the validity of the *in vitro* KD results, CM-specific *in vivo* KD in P7 mice was targeted (FIG. 9B) using the AAV9-TNNT2-eGFP RNAi delivery system. Following injection of either AAV9-TNNT2-eGFP-*Sghrt* KD in P7 mice, hearts were harvested at P14. *Sghrt*-KD hearts indeed bore significantly more dedifferentiated CMs (DAB2⁺) (FIG. 9C,D).

Next, it was confirmed that DAB2⁺ CMs possessed the typical properties of dedifferentiated CMs with cell size and number of nuclei. DAB2⁺ CMs (n=15) were smaller than their neighbouring DAB2⁻ CMs (n=45) (FIG. 9E). Among all 478 DAB2⁺ CMs, 94.98% were mononucleated (FIG. 9F, right panel and FIG. 9G) and only very few of them were binucleated (FIG. 9F, left panel). These results indicated that KD of *Sghrt* resulted in CM dedifferentiation in neonatal mouse.

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Sghrt knockdown results in increased dedifferentiation of adult mouse cardiomyocytes and functional recovery after TAC pressure overload

Single nuclear RNA-seq on cardiomyocytes isolated from healthy and failing adult mouse left ventricles revealed *Sghrt* to be highly upregulated in a subpopulation of cardiomyocytes (FIG. 10A). This subpopulation of cardiomyocytes was unique because of their expression pattern in the genes encoding for cell cycle activators and inhibitors. Through gene network analysis, *Sghrt* stood out as a putative key regulator for the cardiomyocyte stress gene programme. Therefore, it was decided to carry out *Sghrt* knockdown in the TAC pressure overload HF model.

Four weeks after TAC surgery, mice were injected with AAV9 to deliver 2 unique *Sghrt* RNAi reagents of either *Sghrt*-RNAi-#1, *Sghrt*-RNAi-#2, or the control LacZ sequence. Six weeks after AAV9 injection, mice were assessed by echocardiography before their hearts were harvested and assessed for CM dedifferentiation by immunofluorescence staining (FIG. 10B). Mice receiving LacZ as control (n = 4) showed the expected significant deterioration in cardiac

function (EF% and FS%) from TAC-surgery, compared to Sham-operated mice (n =3). Cardiac function of mice from *Sghrt*-RNAi-#2 group was significantly rescued. No significant difference was found between the EF% and FS% of *Sghrt*-RNAi-#1 and control groups (FIG. 10C), possibly due to the larger variation within groups and small sample size.

Importantly, more DAB2⁺ cardiomyocytes (FIG. 10D) per heart section were found, 6 weeks after *Sghrt* KD, compared to control mice (FIG. 10E), where DAB2 has been proposed as a *bona fide* marker of cardiomyocyte dedifferentiation. Consistent with the hallmark of cardiomyocyte dedifferentiation in DAB2-positive cells, DAB2⁺ CMs (n = 36 CMs) were overall smaller than the DAB2⁻ CMs (n = 108 CMs) (FIG. 10F), and they were also predominantly mononucleated (FIG. 10G-H). The results thus suggest that *Sghrt* KD improves heart function of failing mouse heart, associated with enhanced dedifferentiation of adult mouse cardiomyocytes in *Sghrt*-inhibited hearts.

SGHRT micropeptide is required for fatty acid utilization in cardiomyocytes

To study function of SGHRT micropeptide in cardiomyocytes, ATG (TCG in reverse strand)-mutated human embryonic stem (ES) cell clones were generated using CRISPR-CAS based editing technology to change A or C into G or T (Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 533, 420-424, doi:10.1038/nature17946 (2016); Gaudelli, N. M. et al. Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage. *Nature* 551, 464-471, doi:10.1038/nature24644 (2017).) DNA sequencing confirmed that 2 A-to-G (BE19-4 and BE19-10) and 2 C-to-T (BE20-1 and BE20-2) based editing ES clones were successfully generated (FIG. 11A). The Q-PCR data indicated there was no significant change in SGHRT RNAs in BE ES clones compared to WT ES (FIG. 11B), suggesting that changing ATG site in SGHRT DNA did not affect RNA expression. These BE ES clones were then differentiated into cardiomyocytes (CMs) and ATP measurement was performed on these cardiomyocytes treated with or without Fatty Acid (FA). It was

found that WT CMs produce more ATP when treated with FA compared to non-treated condition whereas KO and BE CM clones did not produce more ATP in response to FA treatment (FIG. 11C). Taken together, these results suggested that SGHRT is a mitochondria micropeptide required for FA utilization in cardiomyocytes.

Experimental procedures

Tissue and cell samples

Human heart (Left Ventricle) samples were collected with an ongoing protocol approved by the Papworth(Cambridge) Hospital Tissue Bank Review Board and the Cambridgeshire Research Ethics Committee (UK). Samples were from patients undergoing cardiac transplantation for end-stage ischemic and idiopathic cardiomyopathy. The tissues were frozen and kept in -80°C freezer until being used for mitochondria extraction.

Pig, rat and mouse heart (Left Ventricles) samples were collected from pig, rat and mouse respectively, snapped frozen and kept in -80°C freezer until being used for mitochondria extraction.

Human ES-derived cardiomyocytes were collected from cell culture, snapped frozen and kept in -80°C freezer until being used for extraction.

Extraction of mitochondria from tissue

Tissue was cut into small pieces using tweezer and scissors, then homogenised with a mechanical blender in hypotonic buffer (NaCl 10mM, MgCl₂ 1.5mM, Tris 10mM, adjusted with HCl to pH 7.5). Cells were then ruptured by douncing until approximately 80% of cell nucleus was naked. The cell lysate was then rendered isotonic by adding a concentrated mannitol solution to final concentration of 210mM mannitol, 70mM sucrose, 5mM Tris, 1mM EDTA, adjusted with HCl to pH 7.5.

Intact cells and nucleus were then depleted from the lysate by centrifuging at 1300g for 5 minutes at 4°C and discarding the pellet for three cycles. Mitochondria were then harvested from the supernatant as a pellet by centrifuging

at 8000g for 15 minutes at 4°C, discarding the supernatant, and then resuspending in isotonic mannitol solution for two cycles.

Extraction of membrane protein from mitochondria

5 Organic cell lysis buffer [2-propanol:acetonitrile:hexafluoro-isopropanol:water (70:25:0.56:4.44, by vol) containing 20mM ammonium formate, pH 3.7] was added to the mitochondria pellet at approximately 9:1 volumetric ratio. The lysate was then vortexed for 1 minute and sonicated for 15 minutes in ice water bath. The supernatant obtained after centrifuging at 21,000g
10 for 10 minutes at 4°C was then used for LC-MS.

LC-MS

 An Agilent Infinity II 6550B UPLC-QTOF system was used for LC-MS. Mobile phases for UPLC chromatography were water with 0.1% formic acid and
15 acetonitrile with 0.1% formic acid. Reverse phase separation was performed using a Phenomenex Luna Omega 1.6 um Polar C18 100 A LC Column 100 x 2.1 mm, with linear gradient of 20% to 80% over 20 minutes. The eluent was connected online to the mass spectrometer to acquire mass spectrum over time.

 It will be appreciated by a person skilled in the art that other variations
20 and/or modifications may be made to the embodiments disclosed herein without departing from the spirit or scope of the disclosure as broadly described. For example, in the description herein, features of different exemplary embodiments may be mixed, combined, interchanged, incorporated, adopted, modified, included etc. or the like across different exemplary embodiments. The present
25 embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.

Stem cell maintenance and differentiation

 Human embryonic stem cell line H1 was maintained using mTeSR medium
30 (Stemcell Technologies, 85850) on 1:200 growth factor-reduced Geltrex (Thermo fisher, A1413202) coated tissue culture plates and passaged regularly as cell aggregates every 4-5 days using ReLeSR (Stemcell Technologies, 05872), an

enzyme-free dissociation reagent specific for human pluripotent stem cells). Two days prior to starting differentiation, cells were dissociated using Accutase (Stemcell Technologies, 07922) and seeded as single cells in Geltrex -coated 12-well plates (passage ratio 1:2, between 500'000-600'000 cells). Differentiation was performed following the published protocol by Lian et al. (Lian et al. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/beta-catenin signaling under fully defined conditions. *Nature protocols* 8, 162-175, doi:10.1038/nprot.2012.150 (2013)) with modifications as follows. 10 μ M of CHIR99021 (Stemcell Technologies, 72054) was added on day 0 and left for 24 hours followed by medium change. On day 3, 5 μ M IWP2 (Sigma Aldrich, I0536) was added using 50/50 mix of new fresh medium and conditioned medium collected from each well and left for 48 hours. Culture medium from day 0 until day 7 was RPMI1640 (HyClone, SH30027.01) plus B-27 serum-free supplement without insulin (Gibco, A1895601). From day 7 and onwards, RPMI1640 with B-27 serum free supplement with insulin (Gibco, 17504044) was used and changed every 2-3 days.

Generation of MYH6-GFP reporter line

EGFP cassette with kanamycin selection was inserted into BACs for *MYH6* (RP11-834J17, BacPac) immediately before the initiating Methionine (ATG) using recombineering (Quick & Easy BAC Modification Kit, KD-001, Gene Bridges GmbH). The Tol2 transposon cassette with Ampicilin selection mark was inserted into the loxp site of the BAC in the backbone using recombineering. Ten million H1 cells were cultured in CF1 conditioned medium (20% KO serum replacement, 1 mM l-glutamine, 1% non-essential amino acids, 0.1 mM 2-mercaptoethanol and 8 ng ml⁻¹ of basic fibroblast growth factor in DMEM:F12) for 6 days and dissociated into single cells with TrypLE™ Express (Life Technologies) and electroporated with 20 micrograms of Tol2 transposomes and 100 micrograms of Tol2/EGFP modified Transposon-BACs. After electroporation, cells were re-suspended in conditioned medium with 10 μ M ROCK inhibitor Y276329 (Y27632 (Stemcell technology, 72302). ROCK inhibitor was added for the first 48 hours after electroporation. Fifty μ g/ml geneticin (Gibco, 10131035)

was added for selection of positive clones 72 hours post-electroporation. Fourteen days later after drug selection, single colonies were picked into 24 well plates for expansion. Fluorescent *in situ* hybridization (FISH) using non-modified BACs as probes was carried out to validate the incorporation of BAC construct into genome of ES cells (Cytogenetics Services, Genome Institute of Singapore). Karyotyping was performed to confirm a normal chromosome pattern.

Generation of SGHRT knockout ESC lines using CRISPR/Cas9 knockout

Plasmid pMIA3 (Addgene plasmid # 109399; <http://n2t.net/addgene:109399>; RRID: Addgene_109399) was used for CRISPR/Cas9 mediated KO. Two KO hESC lines were generated using dual single guide RNA (sgRNAs) to remove part of SGHRT. Single guide RNA sequence was designed using cloud based software tools for digital DNA sequence editing Benchling and CRISPOR. 10 μ M sense and anti-sense oligonucleotides were ordered and annealed to generate the 20 nucleotide spacer that defines the genomic target to be modified (VHRT 5'end and 3'end). pMIA3 was digested with BsmBI and sgRNA spacers cloned after human U6 promoter with T4 DNA ligase (New England Biolabs) following manufacturer's instruction. Ligated constructs underwent transformation using RapidTrans™ Chemically Competent Cells (Active motif). Plasmid was extracted from bacterial culture, purified and Sanger sequenced to confirm successful cloning. Prior to hESC targeting, cutting efficiency of pMIA3-sgRNAs plasmids were tested on HEK293T using the EGxxFP plasmid (pCAG-EGxxFP was a gift from Masahito Ikawa, Addgene plasmid # 50716). Best combination of sgRNAs was then used for final targeting of hESCs. The best pair of guides were cloned into a single pMIA3 plasmid digested with NheI & XbaI, using NEBuilder isothermal assembly (New England Biolabs), according to manufacturer's instructions to make the final pMIA3 dual sgRNA plasmid. Human ESCs were dissociated with Accutase (Stemcell Technologies, 07922) and $\sim 1.5 \times 10^6$ cells were re-suspended in 100 μ l P3 Primary Cell kit solution from Lonza (V4XP-3024) and mixed with 10 μ g pMIA3 dual sgRNA plasmid. To transfect hESC, nucleofection was performed using program CM-113 on the 4D-Nucleofector System (Lonza). Cells were then

plated into Geltrex coated 6-well plate with mTeSR medium (Stemcell Technologies, 85850) containing 5 μ M Y-27632. After 2 days in culture, cells were dissociated, FACS sorted for RFP positive cells and collected into a tube containing mTeSR medium (Stemcell Technologies, 85850) with 5 μ M Y-27632. 500 to 2000 cells were plated into wells of 6-well plate containing the above media. Single cell clones were monitored and upon sizeable growth, colonies were picked and passaged. Genomic DNA was extracted for genotyping and screened for successful KO. RT-qPCR was performed to validate KD of SGHRT transcript.

10

Generation CRISPR/CAS9 based editing

Plasmid pMIA19-CBE4 and pMIA20-ABE7 were used for CRISPR/Cas9 mediated Based Editing (BE) to change from ATG to ATA and from ATG to ACG respectively. BE hESC lines were generated using single guide RNA (sgRNAs) to edit from ATG to ATA site or from ATG to ACG of SGHRT. Single guide RNA sequence was designed using cloud based software tools for digital DNA sequence editing Benchling and CRISPOR. 10 μ M sense and anti-sense oligonucleotides were ordered and annealed to generate the 20 nucleotide spacer that defines the genomic target to be modified (VHRT 5'end and 3'end). pMIA19-CBE4 and pMIA20-ABE7 were digested with AarI and spacers cloned after human U6 promoter with T4 DNA ligase (New England Biolabs) following manufacturer's instruction. Ligated constructs underwent transformation using RapidTrans™ Chemically Competent Cells (Active motif). Plasmid was extracted from bacterial culture, purified and Sanger sequenced to confirm successful cloning. Human ESCs were dissociated with Accutase (Stemcell Technologies, 07922) and $\sim 1.5 \times 10^6$ cells were re-suspended in 100 μ l P3 Primary Cell kit solution from Lonza (V4XP-3024) and mixed with 10 μ g plasmid. To transfect hESC, nucleofection was performed using program CM-113 on the 4D-Nucleofector System (Lonza). Cells were then plated into Geltrex coated 6-well plate with mTeSR medium (Stemcell Technologies, 85850) containing 5 μ M Y-27632. After 2 days in culture, cells were dissociated, FACS sorted for RFP positive cells and collected into a tube containing mTeSR medium (Stemcell

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Technologies, 85850) with 5 μ M Y-27632. 500 to 2000 cells were plated into wells of 6-well plate containing the above media. Single cell clones were monitored and upon sizeable growth, colonies were picked and passaged. Genomic DNA was extracted for genotyping and screened for successful base editing. RT-qPCR was performed to validate if there is any change in the expression of SGHRT transcript.

Immunostaining

Cells were fixed in 3.7% formaldehyde for 15 min at room temperature and stored in DPBS. They were permeabilized in 0.2% Triton X-100 for 15 min followed by a pre-blocking step with 2% BSA for 20 min. Primary antibody incubation was performed in DPBS + 10% goat serum (except for Nkx2.5 for which donkey serum was used) overnight at 4 degree and secondary antibody incubation for 2 hours at room temperature. DAPI was included during the final washing step. Antibodies used were cardiac troponin T (Lab Vision, ms-295-P0, mouse, 1:500 dilution), α -DAB2 (Santa Cruz, , rabbit, 1:200 dilution), alexa fluor 594 goat-anti-mouse, alexa fluor 546 goat-anti-rabbit (Life Technology, A-11071), alexa fluor 568 donkey-anti-goat (Life Technologies, A11057).

RNA and DNA isolation

RNA was extracted using Direct-zol™ RNA MiniPrep Kit (Zymo, R2060). Cells were directly lysed using Trizol reagent (Thermo Fisher, 15596026). DNA was purified using PureLink Genomic DNA Mini Kit (Thermo Fisher, K182001). All experiments were performed following the manufacturer's instructions.

PCR and reverse transcription quantitative PCR (RT-qPCR)

DNA or plasmid vector were PCR amplified using Q5 High-Fidelity 2X Master Mix (Bio Labs, M0492S) and target specific primers (IDT) following manufacturer's instructions.

RNA (50-500 ng) was reverse transcribed to cDNA using qScript Flex cDNA Kit (Quantabio, 95049-025) with a combination of random primers and oligo (dT). Subsequently, 1ul of cDNA (1:10) was used to PCR amplify only

specific SGHRT transcripts. The remaining cDNA (1:10) was mixed with PerfeCTa SYBR Green FastMix, low ROX (Quantabio, 95074-05K) and specific primers on a 384-well plate. Real time qPCR was run using ViiA 7 Real-Time PCR System (Applied biosystems). Average Cq was recorded and $\Delta\Delta Cq$ method
5 was used to calculate relative gene expression changes. Expression levels of genes were normalized against two housekeeping genes, *GAPDH* and *PPIA*.

Transverse aortic constriction (TAC) model

In this disclosure, transverse aortic constriction (TAC) in adult mouse was
10 used as the model of heart failure. TAC in mouse is an experimental model for cardiac hypertrophy and heart failure induced by LV pressure overload. In this model, the constriction at aortic arch between left common carotid artery and right common carotid artery initially obstructs the blood pumped from left ventricle (LV), leading to compensated hypertrophy of the heart and a temporary enhancement
15 of cardiac contractility. However, this response to the chronic LV overload becomes maladaptive overtime, which eventually results in cardiac dilatation and HF, accompanied by fibrosis formation within myocardium. Compared to MI model, TAC provides a more gradual time course in the development of heart failure.

20

Evaluating cardiac function in diseased models of heart failure by echocardiography

Echocardiography (echo) detects the cavity and chamber wall of hearts by sound waves to produce live images, and is an important non-invasive
25 experimental method to visualize the cardiovascular structures and evaluate cardiac function in mice and rats. Improved echo instrumentation can provide accurate assessment of LV systolic/diastolic function, chamber size and wall thickness in various mouse models of cardiovascular diseases. In this disclosure, echo was used to track the structural and functional changes in TAC and MI
30 mouse models.

Quantification and characterisation of dedifferentiated CMs by immunofluorescence staining and confocal microscopy

The method of characterising CM dedifferentiation is not as well-established as that of CM proliferation, which can be indicated by markers of different cell-cycle stages. Based on all the existing publications related to CM dedifferentiation, the following criteria has been used to identify it: 1) loss of contractility and electrophysiological properties; 2) disassembled sarcomere or decreased expression level of sarcomeric genes; 3) expression of stem/cardiac progenitor cell markers, among which disabled homolog 2 (*DAB2*) is most commonly used. The first criterion is usually used to judge *in vitro* dedifferentiation of single CM, rather than CMs from heart tissue sections, while the last criterion is quite controversial. *DAB2* is a target of *GATA* transcription factors and its increase may reflect increased expression of *GATA4/6*, a reported regulator of cardiac hypertrophy. Quantitative proteomics of human embryonic stem cells (hESCs), cardiac progenitor cells (CPCs), and cardiomyocytes also identified *DAB2* as crucial cardiac developmental regulator. However, there is no direct evidence showing the change of *DAB2* expression level in CMs over heart development, nor is its role in the dedifferentiation of other cell types. The first publication using *DAB2* to indicate CM dedifferentiation also didn't provide detailed rationale of choosing it as a marker. Therefore, in this disclosure, immunofluorescence (IF) staining and confocal microscopy were used to identify CMs that are immune-positive of *DAB2*, of which the sarcomere would be judged as well-assembled or not based on IF staining of CM sarcomeric gene. Besides, the cell size of *DAB2*⁺ CMs were measured, and the number of nuclei within these CMs were counted, as dedifferentiated cells are generally smaller and have fewer organelles compared to matured cells.

In vivo knockdown (KD) of Sghrt in CMs via adeno-associated virus serotype 9 (AAV9) containing CM-targeting RNA interference system

RNA interference (RNAi) is an approach to silencing target genes by degrading the mRNA, achieved by introducing small double-stranded interfering RNAs (siRNA) into the cytoplasm. Adeno-associated virus (AAV) is an ideal

vector for gene or siRNA delivery due to its low immunogenicity and stable gene expression, and AAV serotype 9 (AAV9) provides global cardiac gene transfer in mouse and rat, which is superior to other serotypes. To achieve knockdown (KD) of *Sghrt* in CMs in mouse, AAV9 containing cardiac troponin T (cTnT)-promoted and GFP-tagged siRNA targeting *Sghrt* (AAV9-cTnT-eGFP-RNAi) were injected
5 into chest cavity of the mice in the dose of 5×10^{13} vector genomes (vg)/kg.

Studying the effect of Sghrt KD on CM dedifferentiation and heart function in TAC and MI mouse model

10 To investigate whether *in vivo* KD of *Sghrt* induces CM dedifferentiation and rescues cardiac function in diseased models of HF, the mice were given injection of AAV9-cTnT-eGFP-RNAi 4 weeks after TAC surgery and immediately after MI surgery, respectively. AAV9-cTnT-eGFP-LacZ was injected into TAC or MI mice as control. Weekly echo was applied afterwards to track the cardiac
15 function of the mice, including the LV ejection fraction (EF) and fraction shortening (FS). 10 and 14 weeks after TAC surgery, the hearts were harvested and sectioned for histological study and immunofluorescence staining of *DAB2*, cardiac troponin I (*cTNI*) and wheat germ agglutinin (WGA) to distinguish cell membrane. The co-localization of *DAB2* and *cTNI* signals were assessed by z-
20 stacking function of confocal microscopy. The number of *DAB2*⁺ and *cTNI*⁺ cells in each heart section were counted, and the size of the *DAB2*⁺ CMs and 3 neighbouring *DAB2*⁻ CMs were measured and analysed using ImageJ. Similarly, 4 weeks after MI surgery, the hearts were harvested for the same analysis above. Further, to study the effect of *Sghrt* KD on CM dedifferentiation in postnatal
25 mouse, 7-day-old (P7) mice were given injection of AAV9-cTnT-eGFP-RNAi or AAV9-cTnT-eGFP-LacZ, followed by harvesting and downstream analysis 7 days after injection.

ATP quantification

30 ATP quantification in human ES-CMs were perform using ATP Determination Kit (A22066) following instructions provided in the kit.

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CLAIMS

1. An isolated polypeptide encoded by *Sghrt*.
- 5 2. The polypeptide according to claim 1, wherein the polypeptide comprises a mitochondrial polypeptide.
3. The polypeptide according to claim 1 or claim 2, wherein the polypeptide shares at least 75% sequence identity with any one of
10 SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 18, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25.
4. The polypeptide according to any one of claims 1-3, wherein the
15 polypeptide comprises a micropeptide.
5. The polypeptide according to any one of claims 1-4, wherein the polypeptide comprises a homo-oligomer.
- 20 6. A method of assessing heart function in a subject, the method comprising:
determining an expression level of the polypeptide according to any one of claims 1-5 in a sample obtained from the subject;
comparing the expression level to a reference expression level,
25 wherein if the expression level exceeds the reference expression level, the subject is considered to have impaired or deteriorated heart function.
7. The method according to claim 6, wherein the reference expression
30 level comprises an expression level of the polypeptide in a healthy population.

8. The method according to claim 6, wherein the reference expression level comprises an expression level of the polypeptide in an earlier sample obtained from the subject.
- 5 9. The method according to any one of claims 6-8, the method further comprising administering to the subject an inhibitor of the polypeptide.
10. A method of treating impaired heart function in a subject, the method comprising:
- 10 inhibiting an expression of the polypeptide according to any one of claims 1-5 in the subject.
11. A method of identifying a potential drug for treating impaired heart function, the method comprising:
- 15 determining a first expression level of the polypeptide according to any one of claims 1-5 in a cell;
- exposing the cell to a drug candidate; and
- determining a second expression level of the polypeptide after the exposure,
- 20 wherein if the second expression level is lower than the first expression level, then the drug candidate is identified as a potential drug for treating impaired heart function.
12. An inhibitor of the polypeptide according to any one of claims 1-5.
- 25 13. The inhibitor according to claim 12 for use in therapy.
14. The inhibitor according to claim 12 for use in treating impaired heart function.
- 30 15. Use of the inhibitor according to claim 12 in the manufacture of a medicament for the treatment of impaired heart function.

16. The method, the inhibitor or the use according to any one of claims 6-11 and 14-15, wherein the impaired heart function is selected from the group consisting of: myocardial infarction, heart failure, coronary artery disease, narrowing of the arteries, heart attack, abnormal heart rhythms, arrhythmias, heart failure, heart valve disease, congenital heart disease, heart muscle disease, cardiomyopathy, pericardial disease, aorta disease, marfan syndrome, genetic cardiomyopathy, non-genetic cardiomyopathy, heart hypertrophy, pressure overload-induced heart dysfunction, and damaged heart tissue.
17. A pharmaceutical composition comprising the inhibitor according to claim 12, and a suitable carrier, adjuvant, diluent and/or excipient.
18. A vector comprising a polynucleotide sequence encoding for the polypeptide according to any one of claims 1-5 or the inhibitor according to claim 12.
19. A host cell transfected with the vector according to claim 18.
20. A transgenic non-human subject comprising a polynucleotide construct encoding for the polypeptide according to any one of claims 1-5.
21. The polypeptide according to any one of claims 1-5 coupled to a detectable label.
22. A method of dedifferentiating and/or proliferating a heart cell, the method comprising:
inhibiting the expression of the polypeptide according to any one of claims 1-5 in the heart cell.
23. A cell produced by the method according to claim 22, or progenies or cell derivatives thereof.

24. An isolated polynucleotide encoding for the polypeptide according to any one of claims 1-5.